THE ROLE OF LANDSCAPE IN MEDIATING BIODIVERSITY AND ECOSYSTEM SERVICES TO AGRICULTURE

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THE ROLE OF LANDSCAPE IN MEDIATING BIODIVERSITY AND ECOSYSTEM SERVICES TO AGRICULTURE

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Currently more than 40% of earth's terrestrial surface is devoted to agriculture and continued agricultural expansion and intensification is the leading cause of biodiversity loss worldwide. Yet, many of the services upon with agriculture relies, such as biological pest control and pollination, are provided by diverse communities of beneficial insects. Several studies have documented declines in the diversity and abundance of pollinators and natural enemies in landscapes with high agricultural cover. However, we lack a framework for predicting which species are most threatened by agricultural intensification and which are likely to persist. Additionally, studies documenting negative effects on ecosystem services and crop yield have been rare. Therefore the ecological but also the economic impact of land use intensification remains unclear. Here, I investigate the effects of landscape simplification due to agricultural intensification on both pollinator and natural enemy communities providing services to fruit farms in New York State. In each study, I evaluate effects on community composition, ecosystem service delivery and ultimately crop yield. My results reveal that landscape simplification is associated with a loss in abundance, species richness and functional diversity from beneficial insect communities. Loss of species from communities was not random but rather mediated by traits that are often similar among closely related species. As a result the evolutionary history represented within communities in highly agricultural landscapes was 200 million years less than communities in more diverse landscapes.

For bees, functional and phylogenetic diversity better predicted pollination services and crop yield compared to species richness alone. Overall, both pollination services and biological control were negatively influenced by agricultural intensification. However, I also explore one landscape and one local scale strategy to recover community diversity and ecosystem services. At the landscape scale, a diversity of crops emphasizing complementarity in bloom time can promote spillover of pollinators from mass flowering crops like apple into consecutively blooming crops such as strawberry. At the local scale, wildflower plantings bordering crops can support diverse pollinator communities but were most effective when implemented in landscapes with intermediate cover of natural habitats. Importantly, pest populations were higher in plantings with an adjacent wildflower border at sites with the least and most natural habitat cover. Wildflower planting did little to enhance biological control of pests. These findings indicate that local habitat enhancements can have costs, but by targeting locations for establishment these costs can be minimized. By understanding the ecology agricultural ecosystems, we can conserve biodiversity while at the same time promoting crop productivity.

BIOGRAPHICAL SKETCH

Heather grew up in the fields and forests of central Pennsylvania. From early on she was fascinated by nature. She was constantly asking her parents why the different critters she kept in shelves full of aquaria behaved, or looked a certain way. It was only natural that this curiosity led her to pursue a career in science.

Before coming to Cornell, Heather studied how land management practices impact white-footed mice populations and the prevalence of the Lyme's disease while working on her BS at Ursinus College. She also worked in the Lab of Ellen Dawley at Ursinus where she explored the function microglial cells in repairing damaged tissues during the process of limb regeneration in salamanders. Before starting graduate school, Heather spent a summer working at the Penn State Center for Statistical Genetics. Heather has been particularly active in advocating for ecosystem service providers, giving talks based on her dissertation research at more then 35 venues including both grower extension services and public science outreach. In her free time, Heather runs a small organic farm with her husband, producing vegetables and raising chickens, ducks turkeys, pigs, and dairy goats.

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I am privileged to have worked with so many brilliant graduate students and post docs. All of my best ideas have come from discussions in the Pollinator and Plant Insect Interactions groups. I am so proud of the community that we have built as students in entomology. It is easier to leave knowing that there are so many leaders striving to make our department even better.

Much of the data in the following chapters could not have been collected and processed without the help of many exceptional undergraduate research assistants including Ellie McCabe, Alena Hutchinson, Miles Renauld, Nolan Amon and Erin Krichilsky. I am also grateful to the farmers who were so generous with their time and their land. I am particularly thankful to those growers who shared their wisdom and advice as I started my own farming endeavors.

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Introduction

Agricultural lands currently cover 40% of the earth's terrestrial surface (Foley 2005) and continued agricultural intensification is the primary threat to biodiversity worldwide (Newbold *et al.* 2015). Yet many of the services upon with agriculture relies, such as biological pest control and pollination, are provided by diverse communities of beneficial insects. Diversity of beneficial insect communities provides resilience to disturbances and maintains the capacity to adapt to future changing environments (Cardinale *et al.* 2012).

The ecosystem services provided by these communities are not easily replaced by external inputs. Despite honey bee management, fruit set and seed set is positively correlated with wild bee abundance across crops and cropping systems around the globe (Garibaldi *et al.* 2014). Perhaps due to limitation in pollination services by wild bees, yield growth has been slower and more variable in pollinator dependent crops compared to pollinator independent crops (Garibaldi *et al.* 2011). Similarly, despite high pesticide usage, yield losses still occur for many crops due to pest outbreaks (Oerke 2006) and pesticide use does not consistently decrease yield losses (Bommarco *et al.* 2011). Therefore, understanding the factors that regulate beneficial insect communities and the delivery of ecosystem services is critical for ensuring agricultural sustainability and food security into the future.

Although many studies have documented the pervasive negative effects of landscape simplification on beneficial insect communities few have measured the effect on yield.

Therefore the literature relating landscape to community diversity and diverse communities to increased yield have largely remained disconnected. It is unclear if landscape simplification results in yield losses. The effect of landscape mediated through ecosystem service providers may be diffuse or many have contrasting effects on different ecosystem service providing groups. Crops may not be pollen limited or may tolerate high levels of damage without negative effect on yield. Alternatively, local management practices or environmental conditions (ie. soil quality) may have a greater impact on crop yield. Nevertheless, the implication in most studies of beneficial insect communities in agriculture is that practices that enhance their abundance and diversity will create a win-win; achieving increases in both conservation and crop productivity. Studies that specifically evaluate the links between landscape structure, ecosystem services, and crop yield are therefore necessary in order to inform policies that target conservation on working lands.

In **Chapter 1**, I explore the influence of landscape on the success of a mixed classical-conservation biological control complex against the generalist pest, *Lygus lineolaris*, in strawberry, *Fragaria x ananassa*. The primary natural enemies of L. lineolaris include an introduced parasitoid, *Peristenus digoneutis* along with a complex of native parasitoids in the same genus, *Peristenus pallipes* sp group. Interestingly we, found no evidence of parasitism by the native species, perhaps representing the final point in a trend of species functional replacement documented by prior studies in the same system (Day *et al.* 1990; Tilmon & Hoffmann 2003). Parasitism rates by the classical biological control agent were highest in landscapes with the greatest cover of forest and open natural

habitat cover. Natural land cover were negatively associated with pest densities and farms with greater proportions of semi-natural habitats in the surrounding landscape had overall higher yields than farms in more agriculturally dominated landscapes.

In agriculturally dominated landscapes, yield losses may also due to decreased pollination services. In **Chapter 2**, I evaluate the role of wild bees in providing pollination services to strawberry and how landscape composition influences wild bee communities. Wild bees comprise 93% of the strawberry flower visitors and wild bees were equally efficient pollinators as honey bees. The cover of agricultural in the surrounding landscape was negatively associated with both abundance and species richness of wild bees. For strawberry, abundance of wild bees but not honey bees or species richness was positively correlated with fruit yield.

In **Chapter 3**, I investigate how loss of species occurs with respect to the bee phylogeny. Phylogenetic diversity metrics integrate many dimensions of functional diversity by quantifying the shared evolutionary history of a community (Cadotte *et al.* 2010; Srivastava *et al.* 2012). Loss of species from communities in high agriculture landscapes was not random but rather mediated by traits that are often similar among closely related species. As a result the evolutionary history represented within communities in highly agricultural landscapes was 200 million years less than communities in more diverse landscapes. Loss of functional and phylogenetic diversity better predicted pollination services and crop yield compared to species richness alone.

At the landscape scale, policies to promote conservation should protect the remaining natural habitat fragments present in highly agricultural landscapes. To some extent, diversification of cropping systems can increase the heterogeneity of the landscape and provide complementarity and continuity of resources for beneficial insect populations (Schellhorn, Gagic & Bommarco 2015). In **Chapter 4**, I show that bloom of mass flowering crops can lead to pollinator spillover and increased yield in consecutively blooming crops independent of landscape context (Grab *et al.* 2017).

The effectiveness of local practices to promote biodiversity and ecosystem services is expected to be dependent on landscape context (Tscharntke *et al.* 2005). In **Chapter 5**, I present the first experimental evidence that the effectiveness of perennial wildflower crop borders for increasing both pollination and biological control is landscape dependent. In accordance with the intermediate landscape hypothesis, Increases in pollination services were greatest in landscapes with intermediate cover of natural habitat. However, the addition of resources into agriculturally dominated landscapes may also benefit pest populations, particularly in landscapes where the abundance of natural enemies was the least improved.

It is clear that landscape scale agricultural simplification is costly both in terms of biodiversity conservation and crop productivity. The studies presented in the following chapters detail these costs and importantly, suggest practices and policies that are likely to benefit both conservation and agriculture.

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CHAPTER 1

Title:

Landscape simplification reduces classical biological control and crop yield

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Abstract:

Agricultural intensification resulting in the simplification of agricultural landscapes is known to negatively impact the delivery of key ecosystem services such as the biological control of crop pests. Both conservation and classical biological control may be influenced by the landscape context in which they are deployed; yet studies examining the role of landscape structure in the establishment and success of introduced natural enemies and their interactions with native communities are lacking. In this study, we investigated the relationship between landscape simplification, classical and conservation biological control services and importantly, the outcome of these interactions for crop yield. We showed that agricultural simplification at the landscape scale is associated with an overall reduction in parasitism rates of crop pests. Additionally, the introduced parasitoids appear to have competitively excluded the native parasitoids in crop habitat irrespective of agricultural landscape simplification. Pest densities in the crop were lower in landscapes with greater proportions of semi natural habitats. Furthermore, farms with less semi-natural cover in the landscape and consequently, higher pest numbers, had lower yields than farms in less agriculturally dominated landscapes. Our study demonstrates the importance of landscape scale agricultural simplification in mediating the success of biological control programs and

highlights the potential risks to native natural enemies in classical biological control programs against native insects. Our results represent an important contribution to an understanding of the landscape-mediated impacts on crop yield which will be essential to implementing effective policies that simultaneously conserve biodiversity and ecosystem services.

Keywords: Agricultural landscape simplification, Ecosystem services, Biological control, Crop yield, Niche compression

Introduction:

Globally, conversion of natural lands to agricultural uses is one of the greatest threats to biodiversity and ecosystem function. Over the past century, the intensification of agricultural production practices has resulted in the simplification of agricultural landscapes and a decrease in the abundance and diversity of farmland plant, bird and insect communities (Symposium & Diversity 2010; Chaplin-Kramer *et al.* 2011; Fischer *et al.* 2011; Batáry *et al.* 2012; Tscharntke *et al.* 2012; Lindborg & Eriksson 2014). In many cases, the species lost in simplified agricultural systems are important providers of ecosystem services including pollination (Steffan-Dewenter, Münzenberg & Tscharntke 2001; Garibaldi *et al.* 2011) and biological control of pests (Chaplin-Kramer *et al.* 2011; Rusch *et al.* 2016). However, even in areas where agricultural practices are locally intensive, structural complexity at the landscape scale can allow for the maintenance of diverse communities of ecosystem service providers (Tscharntke *et al.* 2005; Gámez-Virués *et al.* 2015; Park *et al.* 2015). Thus, understanding the role of

landscape structure in mediating the delivery of ecosystem services is a critical step towards designing agricultural systems that capitalize on ecological processes to increase the quantity and stability of crop yields (Bommarco, Kleijn & Potts 2013).

Although beneficial insects are often negatively impacted by landscape simplification (Thies, Steffan-Dewenter & Tscharntke 2003; Bianchi, Booij & Tscharntke 2006; Chaplin-Kramer *et al.* 2011), pest populations generally benefit from reduced top-down control from natural enemies and reduced bottom-up control due to greater concentrations of crop host plants (Root 1973). In order to decrease pest pressure in agroecosystems, ecologically based management practices have focused on increasing the diversity and abundance of natural enemies. This can be achieved through conservation biological control, which aims to promote naturally occurring enemies, or by classical biological control in which exotic natural enemies are imported and released. Landscape composition is likely to influence the effectiveness of both classical and conservation biological control programs (Gurr & Wratten 1999; Landis, Wratten & Gurr 2000). Many studies have shown effects of landscape composition on natural predator and parasitoid assemblies (Bianchi, Booij & Tscharntke 2006; Chaplin-Kramer *et al.* 2011; Shackelford *et al.* 2013; Veres *et al.* 2013); yet, we are aware of only a few studies of landscape effects on the success of non-native biological control agents (Gardiner *et al.* 2009; Woltz, Isaacs & Landis 2012). Similar to native natural enemies introduced biological control agents are likely to benefit from landscapes with overwintering habitat, floral resources and host reservoirs outside of the cropping system. For example, Pickett et al. (2009) credit the successful establishment of *Peristenus relictus* for the control of *Lygus hesperus* in California to the presence of

wild vegetation in field margins which may have provided alternative hosts and floral resources.

Landscape structure may also mediate the interactions between native and introduced natural enemies (Bowers & Dooley 1991; Didham *et al.* 2007). Exotic biological control agents can be expected to be more effective than native enemies in agricultural systems since they are introduced only when it is perceived that natives provide insufficient control of pest populations. Greater attack rates of the introduced enemy on the pest may lead to competition resulting in niche compression of the native enemies (MacArthur & Wilson 1967; Dickman 1986). Natural habitat remnants in agricultural landscapes may therefore serve as refuges, supporting populations of native enemies that spill over into crop habitats. Because landscapes with lower agricultural land cover tend to have more of these semi-natural refuge habitats, pest control is predicted to be greatest in these landscapes, as complementarity between native and introduced enemies is maximized (Cardinale *et al.* 2003).

Although landscape structure can impact the dynamics of the interactions between native and introduced enemies and their ability to provide biological control services, no studies have explicitly taken a landscape approach to evaluating the success of a classical biological control program. Furthermore, relatively few studies have linked landscape mediated changes in natural enemy communities to changes in pest populations or more importantly to crop yields (Chaplin-Kramer *et al.* 2011; Liere *et al.* 2015); although, demonstrating this link will be critical in designing and implementing ecologically informed agricultural practices (Bommarco, Kleijn & Potts 2013). Using the ubiquitous crop pest, *Lygus lineolaris,* and its complex of native and introduced

parasitoids, we investigated the relationship between landscape scale agricultural simplification, classical and conservation biological control services and crop yield. We predicted that increasing proportions of agriculture in the landscape would lead to decreased attack rates on the pests, and increased pest densities. Therefore, crop yields on farms in simplified landscapes are expected to be lower than on farms with more natural habitat in the surrounding landscape.

Materials and methods:

Study area and sites.

The study was conducted in central New York State, USA in the spring of 2012 in commercial strawberry fields. The region is characterized by a diversity of agricultural uses, including dairy, row crop, tree fruits and vegetables with natural and semi-natural areas of deciduous forest, small woodlots and old fields dispersed throughout. Using the natural variation in habitat composition, 11 farms in five counties representing a gradient in agricultural landscape simplification were identified (Figure S1). All farms included in the study were certified organic or used low intensity management practices. Individual field sites on each farm were comprised of established fields of the most commonly grown strawberry variety in the area, Jewel.

Strawberry (*Fragaria x ananassa*) is an excellent system for understanding how landscape influences the impact of native and introduced natural enemies on pest control because the system contains closely related native and introduced natural enemies attacking the same host. The primary pest of strawberry, *L. lineolaris,* is an

economically important pest of many high value fruit and vegetable crops and also field crops throughout the US. Although *L. lineolaris* is a native insect and its primary natural enemies are a complex of native parasitoids, *Peristenus pallipes* and *Peristenus pseudopallipes* (Hymenoptera: Braconidae)*,* the USDA introduced an exotic parasitoid in the same genus, *Peristenus digoneutis,* to the US in the 1980s. The introduction of *P. digoneutis* by the USDA is a well-studied classical biological control program (Day *et al.* 1990, 2003, 2008, Day 1996, 2005; Lachance, Broadbent & Sears 2001; Tilmon & Hoffmann 2003; Carignan *et al.* 2007; Day & Hoelmer 2012)*.,* and parasitism rates achieved by *P. digoneutis* in the field are well above those recorded for native parasitoids (Day 1996).

Estimating pest densities. Each field was sampled for *L. lineolaris* three times approximately 1 week apart representing petal fall, green fruit and ripe stages of strawberry fruit maturation. We were unable to obtain samples from three farms during the petal fall stage and one farm during the green fruit stage. Nymphs are the predominate life-stage during this time period. *L. lineolaris* nymphs were collected from 2 rows along a 20m transect using a backpack vacuum sampling device (Echo ES 230 Shred 'n Vac, Lake Zurich, IL). The device was applied to the foliage 50 times along each row before the contents of the sample were immobilized with CO_2 gas and all *L. lineolaris* nymphs were collected and stored in 95% ethanol.

Parasitism assay. Random samples of 24 nymphs from each sampling period at each site were selected for parasitism assays. In some cases fewer than 24 nymphs were

collected in a sampling period. In this case all collected nymphs for the period were processed. Diagnostic PCR assays were used to simultaneously estimate parasitism rates and parasitoid species identity, as they are faster and more accurate than rearing or dissection (Tilmon *et al.* 2000; Ashfaq *et al.* 2004). DNA from nymphs was extracted using an abbreviated chlorophorm:isoamyl alcohol protocol developed by Tilmon & Hoffmann (2003). DNA extractions along with negative controls were amplified using *Peristenus* species-specific primers (see Appendix S1) as in Gariepy *et al.* (2005). Using this method, species-specific forward primers are combined with a genus-specific reverse primer to amplify a region including ITS1 and ITS2. Presence of an amplicon indicates parasitism and the length of the fragment indicates parasitoid species identity.

Estimating strawberry yield.

To measure the impact of *L. lineolaris* on yield at each site, 30 secondary fruits from eight of the eleven sites were harvested when ripe and weighed. At the three remaining sites grower harvesting prevented us from obtaining fruit samples. A typical strawberry inflorescence is comprised of a single primary fruit (king berry), a pair of secondary fruit, four tertiary fruit, and sometimes additional quaternary fruit. Secondary fruit were used, as they are less prone to frost damage than primary fruit and due to their later development are more susceptible to damage from *L. lineolaris* nymph feeding.

Strawberries are an aggregate accessory fruit comprised of as many as 300 achenes on a primary fruit and 200 on a secondary fruit (Webb *et al.* 1978). *L. lineolaris* nymphs and adults feed on developing achenes leading to a failure in development of

the surrounding tissues. The weight of a fruit is highly correlated with the number of developed undamaged achenes (Webb, Purves & White 1974). Fruits with a high percentage of damaged achenes develop with major malformations that reduce overall yield and marketability (Schaefers 1980).

Landscape analysis: Landscape simplification was assessed by measuring the proportion of land in annual agricultural (row and vegetable crops), perennial agriculture (orchards, vineyards, forage and pasture), forest and open semi- natural covers (fallows, old fields and wetlands) in circular areas around each of the field sites using the 2012 National Agricultural Statistics Service Cropland Data Layer for New York (USDA, 2012) in ArcGIS 10.1 (ESRI, 2012). Urban habitats comprised only 1% of land cover and were therefore not considered in the analysis. Corn, soy and wheat were the dominant annual agricultural land covers (27%) while pasture (20%) and alfalfa (7%) were the dominant perennial agricultural covers. In order to determine which scale best predicted the abundance and parasitism of *L. lineolaris* nymphs, multiple scales with radii of 250, 500, 750, 1000m were classified. In the study region, the proportion of annual agriculture is negatively related to the cover of open semi-natural habitats (cor = -0.76, P = 0.003), perennial agriculture (cor = -0.66, P = 0.018) and forest cover (cor = -0.63, P = 0.026) while open semi-natural cover is positively correlated with the proportion of perennial agriculture (cor = 0.52, P = 0.08) and forest (cor = 0.61, P = 0.035).

Statistical analyses. To determine the landscape scale most predictive of parasitism rates by *Peristenus* wasps as well as the abundance of *L. lineolaris* nymphs, we used the dredge function (R package MuMIn, Barton, 2013) to construct mixed effects models at each scale (250-1000m) using the nlme package (Pinheiro *et al*., 2014). Minimum adequate models for each response variable (parasitism and nymph abundance) were limited to one scale per model based on second order Akaike Information Criterion (AICc). The cover with the lowest AICc score was selected when two or more scales for a cover were equally likely. The sampling period (petal fall, green fruit, ripe fruit) was included in all models as a fixed effect and sampling period nested within farm was included in all models as a random effect. The most predictive scale for each land cover identified in this analysis was then used in further analyses.

The effect of landscape at the most predictive scale on parasitism of *L. lineolaris* nymphs by *Peristenus* wasps was evaluated with a manual hurdle model which first assessed the effect of landscape on the presence or absence of parasitism and then, for sites where parasitism occurred, evaluates the relationship between landscape and parasitism rates. Generalized linear mixed models (GLMM) were fit using the R package glmmADMB (Skaug *et al.* 2016) with the most predictive landscape scale as the predictor variable and sampling period nested within farm as random effects. A negative binomial error distribution was used in the model predicting presence or absence of parasitism while a gaussian error distribution was used in the model predicting parasitism rate.

The association of landscape at the most predictive scale on *L. lineolaris* nymph abundance was evaluated with a generalized linear mixed model with a poisson error

structure. Sampling period, proportion agriculture at the most predictive scale and their interaction were included as fixed effects with a random effect of farm. The relationship between nymph abundance and parasitism rates was also evaluated with a Poisson GLMM with parasitism rate as the response variable and nymph abundance, sampling stage and their interaction as fixed effects with a random effect of farm.

The impact of *L. lineolaris* abundance on strawberry yield was evaluated with simple linear regression for each sampling period separately and for the average abundance between petal fall and green fruit sampling periods as this time period represents the time when strawberries would have been most susceptible to *L. lineolaris* feeding. Additionally, we evaluated the direct effects of landscape simplification on yield using a simple linear model with average fruit weight as a response variable and land cover at the most predictive scale for *L. lineolaris* nymphs.

Results:

A total of 1683 *L. lineolaris* nymphs were collected from strawberry farms in 2012; from which 766 nymphs were assayed for parasitism by *Peristenus* wasps by amplification of species specific DNA fragments. Parasitism rates ranged from 0 – 46.7%. For all nymphs found to be parasitized, only *P. digoneutis* was identified, with no evidence of parasitism by the native wasp *P. pallipes*. Parasitism rates were zero for nearly half of all collection events. Parasitism rates were best predicted by land covers at the smallest scales (Appendix S2). Greater proportions of annual agriculture in the landscape at the 500m scale were associated with a lower probability of observing a parasitism event ($z_{(11)}$ = -1.94, P = 0.052, Fig 1). Across sites where parasitism did

occur, increasing amounts of open semi-natural habitat surrounding farms were associated with an increase in parasitism rates ($F_{(1,7)}$ = 9.38, P = 0.01, Fig 2) independent of sampling period. A competing model with forest cover at the 250m scale (ΔAICc = 0.72) was also associated with increased parasitism rates ($F_{(1,7)}$ = 10.78, P = 0.01).

L. lineolaris nymph abundance was also influenced most strongly by the proportion of semi-natural land covers but at the 750m scale (Appendix S2). Nymph abundance varied across sampling periods ($F_{(1,2)}$ = 85.84, P = 0.01) but was negatively associated with increasing proportions of semi-natural habitats in the landscape across all sampling periods (z = -4.255, P > 0.001; Fig. 3). Parasitism rates were not associated with nymph densities at petal fall and green fruit sampling periods although there was a positive relationship between nymph density and parasitism rates during the ripe fruit stage when *L. lineolaris* abundance was highest (z = 8.74 P > 0.001).

Sites with greater *L. lineolaris* nymph densities at the petal fall and green fruit sampling periods tended to have lower yields than sites with fewer nymphs during these intervals ($F_{(1,6)}$ = 4.61 P = 0.07, Fig. 4). However, there was no effect of nymph density on yield during the ripe fruit sampling period ($F_{(1,6)}$ = 0.13 P = 0.73). Additionally, yield was negatively associated with landscape composition such that farms with greater proportions of semi-natural habitats in the surrounding landscape had overall higher yields than farms in more agriculturally dominated landscapes ($F_{(1,6)}$ = 20.75 P = 0.003, Fig. 5).

Discussion:

Our study reveals that loss of natural habitats within the landscape associated with an increase in annual agricultural land uses has opposing effects on pest and natural enemy populations leading to a decrease in the effectiveness of classical biological control. Parasitism rates of the introduced wasp decreased while pest numbers increased with greater proportions of agricultural land uses in the surrounding landscape. Although the importance of landscape context in mediating pest control services has been widely recognized (Thies & Tscharntke 1999; Bianchi, Booij & Tscharntke 2006; Rusch *et al.* 2013, 2016) our findings reveal that these same ecological processes can impact classical biological control programs. Our results add a further dimension to this body of research by demonstrating that these effects translated into consequences for strawberry yield. Finally, we show dominance of the introduced parasitoid, *P. digoneutis*, and the complete absence of native wasps attacking *L. lineolaris* nymphs in strawberry fields, suggesting that a non-target effect of this introduced parasitoid has been to displace its native congener.

Increases in annual agriculture surrounding strawberry fields was associated with a decrease in the likelihood of parasitism. When parasitism occurred, sites with fewer permanent natural habitats in the landscape had decreased rates of *L. lineolaris* parasitism by *P. digoneutis*. Parasitism rates were best predicted by smaller spatial scale than those that were most important for predicting *L. lineolaris* nymph densities, likely reflecting differences in the normal foraging ranges of *P. digoneutis* and of female *L. lineolaris* as they move from overwintering habitats into crop fields (Khattat & Stewart

1980; Fleischer, Gaylor & Hue 1988; Bancroft 2005). Higher rates of parasitism in landscapes with greater proportions of permanent natural habitats likely reflect the increased availability of resources for parasitoids, such as nectar and availability of *L. lineolaris* population reservoirs outside of the cropping system. Based on detailed studies of the reproductive biology of *P. digoneutis*, Haye *et al.* (2005) report nectar feeding to be instrumental in achieving high rates of parasitism as newly emerged female wasps have very few mature eggs and were not observed to engage in host feeding. Pickett *et al.* (2009) note the presence of weedy field margins as a factor in the successful establishment of closely related *P. relictus* in California strawberry.

In our study, molecular assays revealed that all parasitism events of *L. lineolaris* nymphs were by *P. digoneutis.* No instances of parasitism by the native *P. pallipes* were found despite the fact that previous studies of parasitism rates of *L. lineolaris* in the same region, and in some cases at the same sites, found *P. pallipes* to be ubiquitous (Tilmon & Hoffmann 2003). These results may represent a culmination of the observed trend in reduced parasitism by the native wasps following the introduction and range expansion of *P. digoneutis* (Day 2005). Prior to the establishment of *P. digoneutis*, parasitism rates by *P. pallipes* often reached 25% (Carignan *et al.* 2007). Tilmon & Hoffmann (2003) reported lower parasitism rates by *P. pallipes* in eastern NY strawberries (3.7%) compared to western NY (10.5%) where *P. digoneutis* was less well established. More recently, parasitism rates of *L. lineolaris* in NJ strawberry averaged 30%, with up to 98% of parasitism events by *P. digoneutis* (Day & Hoelmer 2012). Although parasitoid populations may fluctuate from year to year, it is unlikely that *P. pallipes* would be absent from all sites surveyed across the region in any given year.

This result, combined with evidence from the literature of declines in the native species since the introduction of the non-native, supports the hypothesis of competitive exclusion.

Classical biological control has traditionally been considered as a safe and effective means of pest control, particularly for invasive pests. However, due to the greater number of pre-existing interactions between species, classical biological control of native insects is more risky than for non-natives as loss of native enemies is more likely. In the northeast, competition between introduced *P. digoneutis* and native *P. pallipes* appears to have led to the competitive exclusion of the native parasitoid in strawberry agroecosystems. Similar non-target effects of introduced parasitoids have been reported in other systems (Bennett 1993). Loss of alternative natural enemies from the system is expected to result in a reduction in functional complementarity and the ability of the community to respond to disturbance, potentially leading to a reduction in the resilience of biological control services. Although *P. digoneutis* has become the dominant parasitoid in agricultural habitats, little is known about interactions between *Peristenus* species in non-crop habitats. As a response to competition, *P. pallipes* may have undergone niche compression (MacArthur & Wilson 1967) and stable populations of *P. pallipes* may still persist in other habitats such as natural habitats or fallow fields (Bahlai *et al.* 2015). In this case, functional complementary between parasitoid species attacking *L. lineolaris* in different habitats may maintain higher levels of pest suppression than one species alone. Alternatively, populations of *P. digoneutis* subsidized by host populations in agricultural habitats may spill over into natural habitats resulting in increased pressure on native parasitoid populations (Frost *et al.*

2015). Future studies should therefore evaluate the levels of parasitism and parasitoid community composition of *L. lineolaris* in non-crop habitats. Studies should also focus on evaluating parasitism of *L. lineolaris* in later generations when *P. digoneutis* may compete with later season parasitoids of *L. lineolaris* such as *P. pseudopallipes*.

During the petal fall and green fruit sampling periods, *L. lineolaris* nymph abundances in strawberry were lower in landscapes with greater proportions of natural habitats and higher parasitism rates. These results suggest that *P. digoneutis* may be suppressing *L. lineolaris* populations in these landscapes. Other natural enemies, including predators of *L. lineolaris,* are more likely to be abundant in more complex landscapes as well (Chaplin-Kramer *et al.* 2011). Farms with greater nymph abundances during the petal fall and green fruit sampling periods had smaller fruits at harvest while nymph densities during the last sampling period, when fruit were nearly ripe, were not correlated with fruit weight at harvest. This is to be expected as fruit that has attained final size are less susceptible to *L. lineolaris* feeding damage (Handley & Pollard 1993). These results suggest that farms in simplified agricultural landscapes with greater nymph abundances are likely to experience overall lower yields. Indeed, we found a negative relationship between the proportion of agriculture in the landscape at 500m and the average weight of strawberry fruits at harvest. Although damage from *L. lineolaris* feeding was not directly measured in this study, it is expected that *L. lineolaris* feeding is the primary factor leading to yield loss as damage to fruit from other pests was not observed in any of our samples. Poor pollination may also have contributed to reduced yields on farms in highly agricultural landscapes as strawberry is pollinated by a diverse native bee community, which is negatively impacted by agricultural

simplification (Connelly, Poveda & Loeb 2015). To better understand the relative contributions of pollination and biological control to strawberry yield, we suggest additional studies that independently manipulate pollinator visitation and *L. lineolaris* nymph densities.

Managing ecosystem services to agriculture requires a landscape scale approach (Tscharntke *et al.* 2005, 2012); although, landscape context has rarely been considered in classical biological control programs. Landscape structure is known to influence natural enemy abundance and pest control in other agricultural systems (Bianchi, Booij & Tscharntke 2006; Chaplin-Kramer *et al.* 2011; Rusch *et al.* 2016). As members of higher trophic levels, parasitoids are expected to respond more strongly to landscape context than their hosts (Thies, Steffan-Dewenter & Tscharntke 2003) since parasitoids with small foraging ranges and those that require alternative host and floral resources are filtered from agriculturally simplified landscapes (Gámez-Virués *et al.* 2015). Although landscape mediated changes in natural enemy communities and herbivore densities are expected to translate into lower crop damage and greater yields in complex landscapes, few studies have evaluated either of these outcomes. Thus, our results represent an important contribution to an understanding of the landscape-mediated impacts on crop yield which will be essential to implementing effective policies that simultaneously conserve biodiversity and ecosystem services. At the landscape scale, policies that promote land use diversity by incorporating natural and semi-natural land uses such as forests, parks, old fields, residential and urban areas, are expected to enhance the delivery of biological control services and support crop productivity in nearby agricultural lands. At smaller scales, it is possible that biological control services

in simplified agricultural landscapes could be improved by the incorporation of hedgerows or flowering strips that provide increased floral diversity at the field scale (Blaauw & Isaacs 2015; Sidhu & Joshi 2016).

Our findings have particularly important implications for the practice of classical biological control. Our results indicate that introduced natural enemies are more likely to be successful if landscape context is considered in their establishment. Of the 34% of classical biological control agents that have established in the period between 1890 and the 1960s, the rate of success was higher among those that were established in more stable compared to disturbed habitats (Hall, Ehler & Bisabri-Ershadi 1980). Our results suggest that even in the absence of native natural enemies, conserving natural habitats within agricultural landscapes will promote biological control and crop yield. If exotic natural enemies must be released in agriculturally simplified landscapes, candidate species with life histories that are less dependent on non-crop habitats should be favored or practitioners should consider supplementing these resources with small-scale features such as hedgerows or wildflower plantings (Pickett *et al.* 2009). Finally, practitioners should take into consideration interactions between introduced and native enemies when developing classical biological control programs, particularly for those against native pests.

Monitoring of *P. digoneutis* since its introduction has allowed for insights into its spread, effect on populations of the target pest and its impact on native insect communities (Tilmon & Hoffmann 2003; Day 2005; Day *et al.* 2008). Although it was initially hypothesized that reductions in *L. lineolaris* populations in source crops like alfalfa would lead to reduced damage in high value crops like strawberry, our study is

the first to report an indirect relationship between high levels of parasitism by *P. digoneutis* and greater yield on strawberry farms. Furthermore, we report a strong negative association between landscape simplification and crop yield. Our study sheds new light on the importance of landscape context in mediating the success of classical biological control programs.

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Data Accessibility

Data associated with this manuscript have been deposited in the Dryad Digital Repository, doi: 10.0561/dryad.XXXX.X

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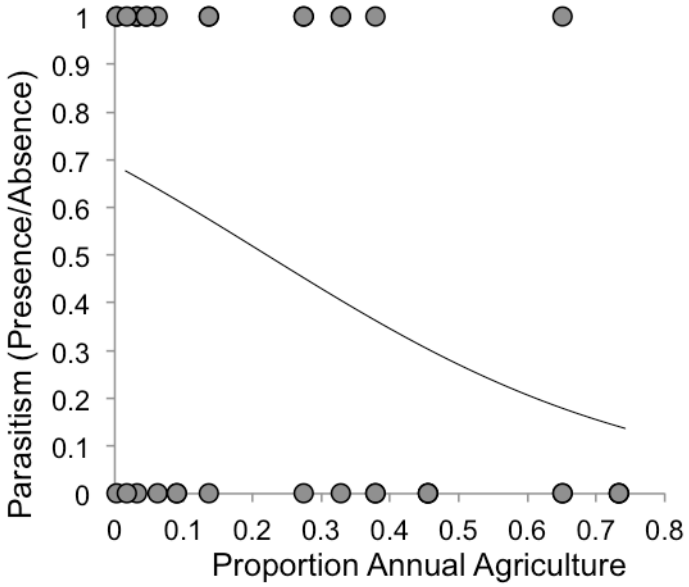

Figure 1.1. Likelihood of parasitism of *L. lineolaris* nymphs by *P. digoneutis* within a given sampling date decreases as a function of the proportion of annual agricultural land use at 500m surrounding the sampling location within each strawberry field.

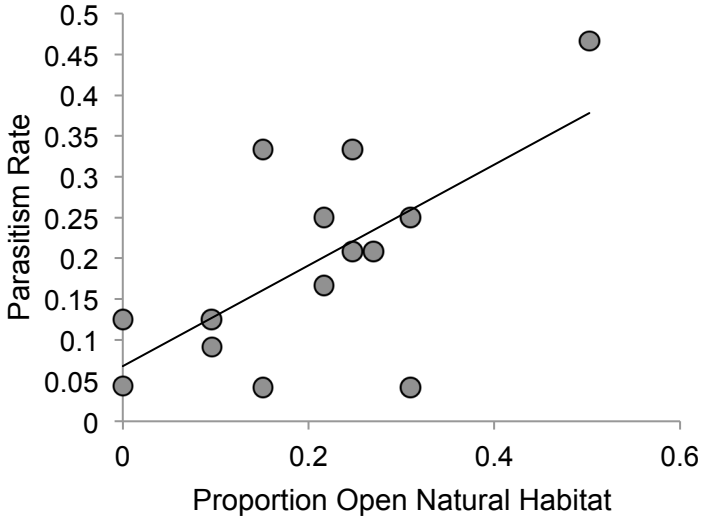

Figure 1.2. Parasitism rates of *L. lineolaris* nymphs by *P. digoneutis* are positively related to the proportion of open semi-natural habitats at 500m surrounding the sampling location within each strawberry field.

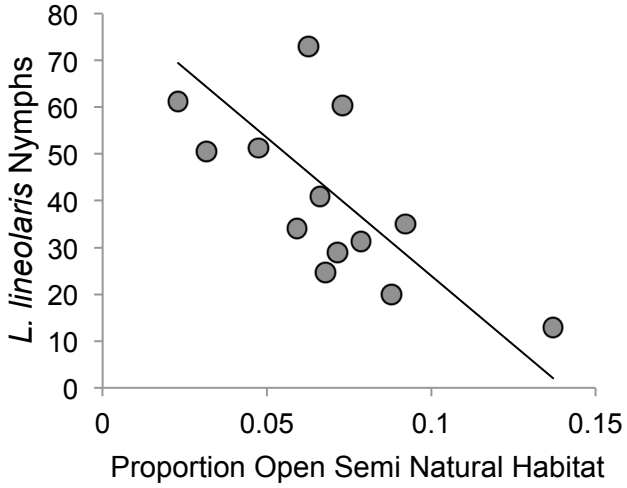

Figure 1.3. Average number of *L. lineolaris* nymphs collected from strawberry fields decrease with respect to the proportion of open semi-natural habitats at 500m surrounding the sampling location within each strawberry field.

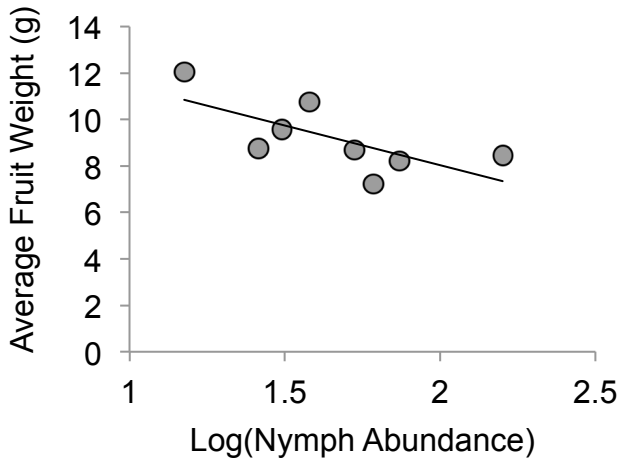

Figure 1.4. Average strawberry fruit weight is negatively related to the log of *L. lineolaris* nymph abundance in the time interval including the petal fall and green fruit sampling periods.

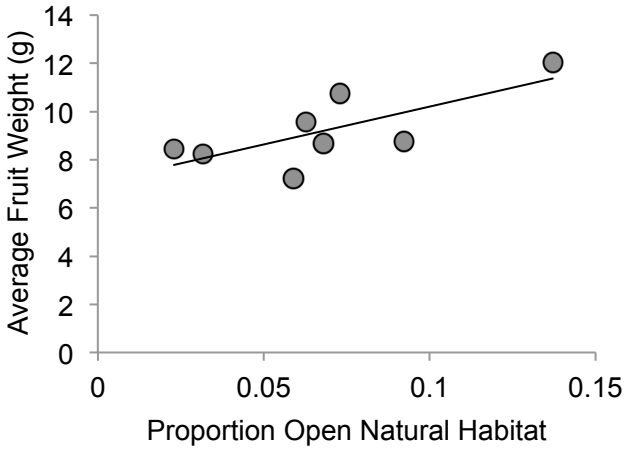

Figure 5. Average weight of strawberry fruits is positively related to the proportion of agricultural land use at 500m surrounding the sampling location within each field.

Table 1.S1. Species specific forward primers and genus specific reverse primers as in Gariepy *et al*. (2005).

Primer	Primer Sequence	Fragment size
Per R1	5'-ACAAGGTTTCCGTAGGTG-3'	---
dig F1096	5'-GAACATAAAAACTTCTTCTACGC-3'	515
pal F517	5'-TAAACTTTGGCCAGATAAATG-3'	1060

PCR conditions: 94°C for 2 min, 35X 94°C|45s, 54°C|45s, 72°C|60s, followed by 72°C for 5 min.

1. Gariepy, T.D., Kuhlmann, U., Haye, T., Gillott, C. & Erlandson, M. (2005) A single-step multiplex PCR assay for the detection of European *Peristenus spp.*, parasitoids of *Lygus spp. Biocontrol Science and Technology*, **15**, 481–495.

Table 1.S2. Model selection table for *L. lineolaris* abundance

		Annual Agriculture				Perennial Agriculture				Open Natural Habitat				Forest								
Int	Stage	250m	500m	750m	1000m	250m	500m	750m	1000m	250m	500m	750m	1000m	250m	500m	750m	1000m	df	logLik	AICc	delta	weight
0.115										**1.40**								**4**	**10.93**	**-9.41**	**0.00**	**0.176**
0.098														**0.69**				**4**	**10.57**	**-8.70**	**0.72**	**0.123**
0.064											**1.87**							**4**	**10.19**	**-7.94**	**1.47**	**0.084**
0.019												2.33						4	9.85	-7.26	2.16	0.060
0.021													2.30					4	9.78	-7.13	2.29	0.056
0.192																		**3**	**7.06**	**-5.72**	**3.70**	**0.028**
0.055																	0.47	4	8.49	-4.54	4.88	0.015
0.102															0.40			4	8.47	-4.50	4.92	0.015
0.095																0.36		4	7.92	-3.39	6.02	0.009
0.250		-0.24																4	7.54	-2.63	6.78	0.006
0.242			-0.26															4	7.26	-2.07	7.35	0.004
0.277									-0.26									4	7.14	-1.83	7.58	0.004
0.267								-0.22										4	6.97	-1.50	7.92	0.003
0.228					-0.18													4	6.89	-1.34	8.07	0.003
0.255							-0.17											4	6.79	-1.14	8.28	0.003
0.227				-0.17														4	6.78	-1.12	8.29	0.003
0.187						0.01												4	6.01	0.42	9.83	0.001
0.196	+													0.59				6	10.91	2.18	11.60	0.001
0.308	+																	5	7.58	2.35	11.76	0.000
0.216	+									0.98								6	9.56	4.89	14.30	0.000
0.194	+										1.30							6	9.54	4.91	14.32	0.000
0.180	+												1.52					6	9.49	5.02	14.43	0.000
0.180	+											1.53						6	9.48	5.03	14.45	0.000
0.212	+														0.33			6	8.54	6.91	16.33	0.000
0.185	+																0.37	6	8.35	7.29	16.71	0.000
0.220	+															0.28		6	7.89	8.22	17.64	0.000
0.339	+	-0.22																6	7.82	8.35	17.77	0.000
0.333	+		-0.19															6	7.31	9.37	18.79	0.000
0.334	+				-0.14													6	7.28	9.44	18.86	0.000
0.341	+								-0.11									6	7.17	9.67	19.08	0.000
0.343	+							-0.11										6	7.15	9.70	19.11	0.000
0.328	+			-0.11														6	7.08	9.85	19.26	0.000
0.337	+						-0.08											6	7.06	9.87	19.29	0.000
0.300	+					0.02												6	6.46	11.07	20.49	0.000

Table 1.S3. Model selection table for parasitism rate

		Annual Agriculture				Perennial Agriculture				Open Natural Habitat				Forest								
Int	Stage	250m	500m	750m	1000m	250m	500m	750m	1000m	250m	500m	750m	1000m	250m	500m	750m	1000m	df	logLik	AICc	delta	weight
4.78	+											**-15.71**						**5**	**-226.98**	**466.26**	**0.00**	**0.44**
4.83	+												**-15.80**					**5**	**-227.11**	**466.53**	**0.27**	**0.39**
4.34	+										-10.16							5	-228.77	469.86	3.59	0.07
4.01	+									-6.10								5	-229.74	471.79	5.53	0.03
3.26	+				1.75													5	-231.04	474.38	8.12	0.01
3.30	+			1.54														5	-231.05	474.41	8.14	0.01
3.69	+																	4	-232.77	475.03	8.76	0.01
3.90	+													-2.02				5	-231.38	475.08	8.81	0.01
4.26	+								-1.95									5	-231.59	475.49	9.22	0.00
4.25	+							-1.77										5	-231.83	475.96	9.70	0.00
3.46	+		0.87															5	-231.91	476.12	9.86	0.00
3.88	+														-0.95			5	-232.36	477.03	10.77	0.00
3.58	+	0.37																5	-232.56	477.44	11.17	0.00
3.85	+															-0.59		5	-232.64	477.59	11.33	0.00
3.84	+						-0.41											5	-232.70	477.71	11.45	0.00
3.61	+					0.18												5	-232.73	477.76	11.50	0.00
3.67	+																0.07	5	-232.77	477.85	11.59	0.00
4.56													-13.39					3	-332.70	672.25	205.99	0.00
4.49												-13.01						3	-332.92	672.70	206.44	0.00
4.12											-8.21							3	-334.68	676.22	209.96	0.00
3.85										-4.99								3	-335.41	677.67	211.41	0.00
4.15									-1.92									3	-336.42	679.69	213.43	0.00
3.24					1.43													3	-336.48	679.81	213.55	0.00
3.28				1.24														3	-336.55	679.96	213.70	0.00
3.60																		**2**	**-338.05**	**680.51**	**214.25**	**0.00**
4.13								-1.69										3	-336.84	680.53	214.27	0.00
3.77														-1.63				3	-336.85	680.55	214.29	0.00
3.42			0.65															3	-337.40	681.65	215.39	0.00
3.71															-0.57			3	-337.85	682.55	216.29	0.00
3.49						0.25												3	-337.94	682.73	216.47	0.00
3.53		0.20																3	-337.96	682.79	216.52	0.00
3.72							-0.36											3	-337.97	682.81	216.54	0.00
3.49																	0.34	3	-337.99	682.84	216.58	0.00
3.66																-0.24		3	-338.02	682.90	216.64	0.00

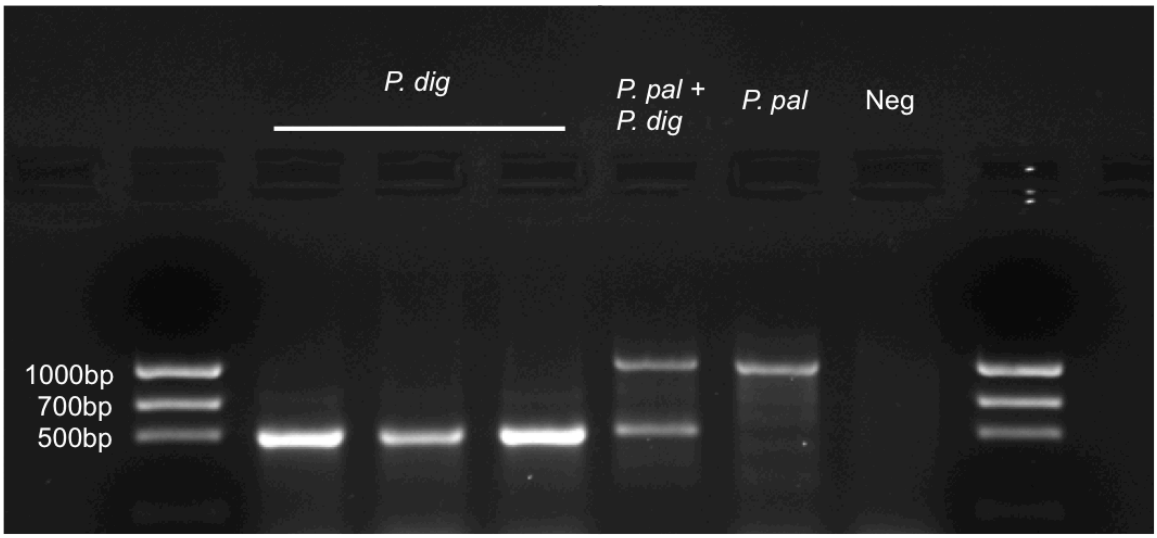

Figure S1. PCR positives for DNA extracts from *Lygus* nymphs parasitized by *P. digoneutis*, *P. pallipes* and both species along with an unparasitized nymph as a negative control.

Figure S2. Map of sampling locations in the Finger Lakes Region of New York State.

CHAPTER 2

Title

Landscape simplification decreases wild bee pollination services to strawberry

Authors

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Highlights

- Strawberry was primarily pollinated by wild bees (93% of collected individuals).
- Landscape simplification reduced abundance and species richness of wild bees.
- Greater pollinator abundance, not species richness, increases strawberry yield.

Abstract

Successful conservation of pollination services depends upon an understanding of the processes that influence pollinator diversity within the landscape, as well as the relationship between pollinator community composition and the provision of services. In this study, the influence of landscape simplification on the composition of pollinator communities and the provisioning of pollination services to cultivated strawberry was evaluated (*Fragaria x ananassa)*. Additionally the relative pollination efficiency for the five most common bee genera visiting strawberry flowers was measured. Pollinators were collected during bloom with pan traps on 14 commercial farms all growing the same strawberry variety in the Finger Lakes Region, NY, USA. The farms were chosen to represent a gradient in the proportion of agricultural land uses in the surrounding landscape from 0.09 to 0.60. Landscapes with greater proportions of agricultural area had lower pollinator abundances and species richness. While yield was positively

correlated with pollinator abundance, species richness had little effect on the level of pollination services. This conclusion was supported by the finding that visitor identity did not explain pollination efficiency in strawberry. Our results show that a high proportion of agricultural land in the landscape negatively impacts wild pollinators and pollination services to strawberry.

Keywords

Ecosystem Services, Landscape Simplification, Pollination, Strawberry, Wild Bees

1. Introduction

Pollinators are essential for the production of more than 70% of globally traded crops (Klein et al., 2007), including a disproportionate amount of the most nutrient dense foods (Eilers et al., 2011). Pollination by insects not only increases crop yield, but also improves aspects of fruit quality, including nutritional value (Brittain et al., 2014) and shelf life (Klatt et al., 2014). Landscape simplification threatens the ability of pollinators to continue providing this essential ecosystem service. Indeed, intensification of agricultural practices and loss of natural habitats have been shown to have a negative impact on both the abundance and species richness of wild bees in agroecosystems (Steffan-Dewenter et al., 2002; Ricketts et al., 2008; Winfree et al., 2009; Kennedy et al., 2013). These landscape-mediated changes in bee community structure are thought to occur through loss of nesting habitat, lack of floral resources and pesticide exposure.

Landscape-mediated reductions in pollinator abundance and diversity are expected to have negative consequences on crop yield. However, only a few studies have explored the impacts of these landscape-mediated changes on yield or seed set (Garibaldi et al., 2011; Holzschuh et al., 2012; Bartomeus et al., 2014; Petersen and Nault, 2014). Yield may be impacted by changes in either the abundance (Bartomeus et al., 2014) or species richness (Klein et al., 2003) of the pollinator community. On the one hand, the abundance of pollinators could be important for achieving high yields in systems where large areas of a single crop species bloom for short periods (Winfree et al., 2007; Garibaldi et al., 2013). On the other hand, the species richness of a community of pollinators is also critical for maintaining yield when different pollinator functional traits are needed to achieve full pollination (Chagnon et al., 1993; Hoehn et al., 2008; Brittain et al., 2013) or under dynamic spatial and temporal conditions (Winfree and Kremen, 2009; Rader et al., 2013). However, it is unclear which of these often-correlated metrics best predict yield depending on context and cropping system.

Pollinator species richness may be particularly important as not all pollinators provide the same level of pollination services to crops. For example, it has been shown that pollination services provided by wild bees exceed those of managed honey bees in many crops (Winfree et al., 2007; Holzschuh et al., 2012; Garibaldi et al., 2013). This pattern may partially be explained by the greater efficiency of wild bees compared to honey bees on a per visit basis although, it has only been explicitly evaluated in a small number of crops (apple = Thomson and Goodell, 2001; blueberry = Javorek et al., 2002; watermelon = Winfree et al., 2007). In many of these comparisons wild bees have been

considered as an aggregate group making it impossible to determine differences in the efficiency between functional groups or species (Chagnon et al., 1993; Albano et al., 2009).

In this study, we explore the effects of landscape simplification on the community of bees providing pollination services to cultivated strawberry (*Fragaria x ananassa*) and determine whether these effects translate into changes in yield. Specifically, we predicted that 1) landscape simplification will negatively impact both the abundance and species richness of wild bees but not honey bee visiting strawberry, 2) farms with lower abundance and species richness of bees will have lower yield, and 3) pollinators visiting strawberry flowers will vary in their per visit pollination efficiency, with some wild bees being more efficient than honey bees.

2. Methods

The study was conducted in the Finger Lakes Region of New York State, USA in the springs of 2012 and 2013 in commercial strawberry fields. Although cultivated strawberry varieties are hermaphroditic and self-fertile, they are regularly visited by pollinating insects, which can increase fruit weight by as much as 40% over wind pollination alone (Zebrowska, 1998; Bartomeus et al., 2014). The pollinator community visiting strawberry flowers is relatively diverse and includes bees, ants, wasps, beetles, flies, and butterflies (Albano et al., 2009). Strawberries are an aggregate accessory fruit comprised of as many as 300 achenes on a primary fruit and 200 on a secondary fruit (Webb et al., 1978). Each achene must be fertilized in order for the surrounding tissue

to develop. Hence, the weight of a fruit is highly correlated with the number of pollinated achenes (Webb et al., 1974) and an average of four visits per flower is required to achieve full pollination and maximum fruit weight (Chagnon et al., 1989). Only fruits with a high percentage of fertilized achenes develop without major malformations that reduce overall yield and marketability. Therefore, the weight of individual fruits can provide an estimate of pollination services.

In the study region, strawberry is grown as a perennial crop. Fields are typically maintained for up to five years after establishment; although, yield generally decreases each year after the first fruiting year. The region is characterized by a diversity of agricultural uses, including dairy, row crop, tree fruits and vegetables with natural and semi-natural areas of deciduous forest, small woodlots and old field dispersed throughout. Using the natural variation in habitat composition, 14 farms in five counties that represented a gradient in agricultural intensification were identified. Farms were separated by a minimum distance of 0.5 up to 109 km. Agricultural intensification was measured as the proportion of land in agricultural uses (all crop categories including forage and pasture) and landscape complexity as the proportion of land in natural uses (forest, wetlands, shrub lands, wildflowers, and fallow) in circular areas around each of the field sites using the 2012 National Agricultural Statistics Service Cropland Data Layer for New York (USDA, 2013) in ArcGIS 10.1 (ESRI, 2012). In order to determine which scale best predicted the abundance and species richness of the bee community, multiple scales with radii of 500, 750, 1000, 2000, and 3000 m were classified. All farms identified for the study were certified organic or used low intensity management

practices. Individual field sites on each farm were comprised of 5 x 5 m experimental plots within already established fields of the most common strawberry variety, “Jewel”, that varied in age from 1 to 5 y. Plots were located two rows from the field edge in order to standardize for edge effects and were comprised of 5 rows with approximately 1 m between row middles.

2.1 Estimating Pollinator Abundance and Richness

The abundance and diversity of pollinators at each site were sampled using five fluorescent yellow and five white pan traps for each farm. The traps were placed on transects with one pair of white and yellow traps in each row of the experimental plot. Westphal et al. (2008) found that pan traps capture a similar number of individuals in comparison to transects while at the same time a greater diversity of the pollinator community is represented. As some bee species may be differentially attracted to white or yellow traps both colors were used to maximize the diversity of the pollinator community sampled (Campbell and Hanula, 2007, Gollan et al., 2011). Each trap contained 250 ml of a 1% Dawn® dish soap solution and was deployed for 72 h during fair weather conditions (warm, sunny days with zero to moderate cloud cover).

The flowers of a strawberry inflorescence bloom in sequential order starting with a single primary flower, followed by two secondary flowers and finally up to four tertiary flowers (Webb et al., 1978). Farms were sampled twice, once starting on May 5th 2012 corresponding to the bloom of primary flowers and again starting May 15th corresponding with secondary flower bloom. After collection, the contents of the pan

traps from each sampling period were strained from the solution, stored in plastic bags and frozen (-20°C) for later analysis. Frozen samples were thawed and all bees were sorted from other arthropods. The bees were then cleaned, dried and identified to species using the DiscoverLife.org keys, and a reference collection of strawberry pollinators. All bees in the genus *Lasioglossum* were identified to species by Jason Gibbs (Michigan State University). Bees from the white and yellow pan traps were pooled and the total abundance and species richness of bees from each sampling period was calculated for each site.

2.2 Yield

To measure the contribution of the pollinator community to yield at each site, 30 inflorescences from each experimental plot were randomly assigned to one of the following three treatments: self-pollination, open-pollination or hand-pollination. Hand-pollination permitted the determination of the relative pollen limitation at each site when compared to the open-pollinated treatment while the self-pollination treatment allowed for estimation of the contribution of self-pollination to yield. Additionally, statistical comparisons of the hand- and self-pollination treatments to landscape variables indicated whether factors other than the abundance or richness of visitors impacted fruit weight. For the purposes of this experiment, treatments were applied only to the two secondary flowers on each inflorescence, as they are less prone to frost damage. Self-pollinated inflorescences were covered with bonded polypropylene filament bags, designed to allow transmission or air, water and 97% of light (AgroFabric®, Pro97). Flower buds were bagged (May10th) and let to flower and fruit in the bag until harvest

(June 4-8th). Open-pollinated inflorescences were bagged at the bud stage; however, the bags were removed when the secondary flowers opened to allow for visitation. Hand-pollinated inflorescences were treated in the same way as flowers in the open-pollination treatment but supplementary pollen from nearby strawberry flowers of the same variety was added to the stigmas of the secondary flowers using a small paintbrush. Open- and hand-pollinated inflorescences were re-bagged after a minimum of three days to control for any effect of the bags on fruit development and to prevent pest damage. The secondary fruits from each pollination treatment were harvested when ripe (approximately three weeks later) and weighed.

2.3 Pollinator Efficiency

To estimate the contribution of different flower visitors to the pollination of strawberry flowers, we observed single visits to virgin secondary flowers and compared the weight of the resulting fruit to that of the other unvisited secondary flower on the same inflorescence. These observations were made on three farms from the larger study in 2012 and repeated again on one farm in 2013. At each farm approximately 150 inflorescences were covered in fine mesh gauze bags during the bud stage and the primary flower was removed. One week later, as the secondary flowers began to open, the bags were removed from the virgin flowers in groups of 3-5. The grouping was observed until a flower visitor alighted on one of the two open virgin secondary flowers of an inflorescence and was observed collecting pollen or nectar. As the visitor left the flower it was captured, marked with an ID number and preserved for later species-level identification. The visited flower was marked with the corresponding ID number and the

entire inflorescence was covered to prevent further visits. When ripe, the fruits from visited flowers along with the paired unvisited fruit from the same inflorescence were collected and weighed.

2.4 Statistical Analyses

Spatial autocorrelation was tested using the Moran's I function from the package "ape" in R (v 3.1.0, R Core Team, 2012; Paradise et al., 2004) and was not found in either the bee abundance or richness data ($p = 0.267$, $p = 0.625$ respectively). To assess the impact of landscape complexity at multiple scales on the abundance and species richness of wild bees, the dredge function (R package MuMIn, Bartoń 2013) was used to construct all possible mixed effects models using the nlme package (Pinheiro et al., 2012). Minimum adequate models were selected based on second order Akaike Information Criterion (AICc). In the models, the proportion agricultural area and the proportion natural area at each of the landscape scales were included in separate models as fixed effects. The sampling period (primary vs. secondary bloom) was included in all models as a fixed effect and farm was included in all models as a random effect.. Either bee abundance or bee richness were included as response variables. Wild bee abundance and honey bee abundance were analyzed separately because although feral honey bees were likely present at the field sites, many of the farms supplemented with managed honey bee colonies and therefore, honey bee abundance was not expected to be related to landscape metrics. Wild bee abundance was log transformed and wild bee species richness was square root transformed to meet

distributional assumptions. At one site, pan traps from the primary bloom period were damaged by farm equipment and were not included in analyses.

To determine the relative contribution of pollinators to strawberry yield across all farms and estimate the level of pollen limitation, differences in the average weight of self-, hand-, and open pollinated fruits at each site were tested using a one-way ANOVA with farm as a random effect followed by a Tukey HSD test using the glht function in the R package Multcomp (Hothorn et al., 2014).

The contributions of bee abundance and species richness to yield were evaluated with separate multiple linear regression models. Experimental inflorescences on four farms were damaged before the completion of the experiment. For that reason, only yield data from 10 farms could be included in the analyses. Separate models were fit with either the weight of self-, open- or hand-pollinated fruit as the response variable. Field age and bee abundance for each sampling period were included as explanatory variables. The abundances of wild bees and honey bees during each sampling period were included in the model as separate explanatory variables. Models were simplified using backwards-stepwise selection. Separate models were fit for bee abundance and bee richness as bee abundance and richness were correlated (*primary* Pearson's $r = 0.71$, $p = 0.032$, $n = 10$; *secondary* Pearson's $r = 0.62$, $p = 0.05$, $n = 10$). Additionally, we evaluated the direct effects of landscape simplification on yield using separate mixed effects models with average fruit weight from each pollination treatment as a response

variable. Field age and the most predictive landscape scale for the earlier analysis were included in each model as fixed effects and farm was included as a random effect.

Differences in pollinator efficiencies between the most common genera were assessed with two-way analysis of variance. Fruit weight was used as the response variable. Genus of visitor, pollination status of the fruit (visited vs. unvisited) and their interaction were included as explanatory variables. To account for the paired study design, flower ID nested within farm and year were included as random factors in the model. Post hoc contrast tests were used to determine pairwise relationships using the glht function(R package Multcomp).

3. Results

A total of 1,075 bees from 65 species were collected; 755 bees from fluorescent yellow pan traps (55 species) and 320 bees from white pan traps (49 species). Within a sampling period, both the abundance and species richness of bees from white and yellow bowls were highly correlated at each site (Abundance *primary bloom* Pearson's r = 0.81, p = 0.007, n = 13; *secondary bloom* Pearson's r = 0.69, p = 0.03, n = 14; Richness *primary* Pearson's r =0.65, p = 0.05, n = 13; *secondary* Pearson's r =0.77, p = 0.009, n = 14). Within a site, neither the abundance nor the species richness during the primary blooming period was correlated with abundance or richness during secondary bloom (*Abundance* Pearson's r = -0.18, p = 0.66, n = 13; *Richness* Pearson's r =0.14, p = 0.71, n = 13). Across farms, the community was dominated by bees in the genus

Andrena (n = 428, 39.8%) followed by *Lasioglossum* (n = 364, 33.9%) and *Augochlorella* (n = 85, 7.9%). Honey bees were the fourth most abundant visitor comprising only 6.7% of collected bees.

Both wild bee abundance and species richness were best explained by the proportion of agriculture at the 1 km scale (Table 1, Figures 1 & 2). At this scale, landscapes in the study ranged in proportion of agriculture from 0.09 to 0.61. As the proportion of agricultural land increased, the abundances and species richness of the wild bee community decreased (Fig. 1 & 2). Although there were only marginal differences in wild bee abundance between the primary and secondary sampling periods, species richness differed between sampling periods (Table 1). The proportion of agriculture at the 750 m scale was also negatively correlated with wild bee abundance (Table 1). There were no effects of landscape simplification or sampling period on honey bee abundance at any landscape scale (Table 1). Interestingly, the proportion of agriculture in the landscape better predicted all response variables than the proportion of natural area at any of the scales examined (Table 1).

When comparing the effects of self-pollination, hand-pollination and open-pollination across all of the farms, the average yield of fruits differed among treatments ($F_{2,20}$ = 11.60, p = 0.0004, Fig. 3). Hand-pollinated flowers achieved the highest yields (mean =10.8g), but were not different from the open pollination treatment (mean = 8.37g, Fig. 3). Both the hand-pollination treatment and the open-pollinated treatment had greater weights than the closed-pollinated fruits (mean= 5.43g, Fig. 3).

Models describing the relationship between the yield of open-pollinated fruit and pollinator abundance showed that low abundances of bees had a negative impact on yield ($F_{3,6}$ = 18.04, p = 0.002, adj. R^2 = 0.86, Fig. 4). The age of the field had a negative impact on fruit weight ($F_{1,6}$= 32.28, p = 0.001), while wild bee abundance in the secondary sampling period showed a strong positive correlation with fruit weight ($F_{1,6}$ = 14.08, p = 0.003). Honey bee abundance also impacted yield, but less so than wild bee abundance, as honey bee abundance in the secondary sampling period had a marginally positive effect on open-pollinated fruit weight ($F_{1,6}$ = 4.19, p = 0.06, Fig. 4). Neither wild bee abundance nor honey bee abundance in the primary sampling period were correlated with the weight of open-pollinated fruits ($F_{1,3}$ = 0.08, p = 0.80; and $F_{1,3}$ = 0.02, p = 0.90 respectively) and were subsequently removed from the final model. There was no effect of bee species richness on yield in either sampling period (*primary* $F_{1,5}$ = 0.02, p = 0.90; *secondary* $F_{1,5}$ = 0.08, p = 0.78) and there was no direct effect of landscape complexity on yield ($F_{1,7}$ = 3.25, p = 0.11). Neither bee abundance nor species richness were related to the weight of fruit in the closed-pollination treatment (*abundance* $F_{5,3}$ = 0.27, p= 0.89; *richness* $F_{3,5}$ = 1.70, p=0.28). In the hand-pollination treatment, fruit weight was not correlated with bee abundance but was marginally related to species richness (*abundance* $F_{4,4}$ = 1.05, p = 0.48; richness $F_{3,5}$ = 4.53, p = 0.07).

Across the genera observed, fruits visited by a bee had higher weights than their paired unvisited fruit ($F_{1,143}$ = 76.07, p < 0.001). On average, visited fruits were 3.53g heavier

than unvisited fruits, representing a 38% increase in yield. However, pollinator identity was found to have only a marginal impact on the magnitude of this yield increase ($F_{5,135}$ = 2.04, p = 0.076) which appeared to be driven by differences between *Apis* and *Ceratina* (Fig. 5). The interaction between pollination status (visited vs. unvisited) and pollinator identity was only marginally significant ($F_{5,138}$ = 2.00, p = 0.08).

4. Discussion

The proportion of agricultural land in the landscape negatively affected wild bee abundance and species richness at both the 750 m and 1 km scales. Bee abundance, but not species richness, was positively correlated with the weight of open-pollinated fruit, suggesting that visitor abundance rather than visitor richness is most important for achieving high yield in this crop. These results are supported by the outcome of the pollinator efficiency experiment, which showed only minor differences in per visit pollinator efficiency between genera.

Our results show that even in a region that is characterized by many small semi-natural areas, agricultural intensification has a negative impact on wild bees. Although previous work in the region did not report landscape effects on the pollinator community visiting watermelon and pumpkin (Winfree et al., 2008), the community of bees pollinating strawberry is comprised of species smaller in average body size, which are thought to be more sensitive to landscape metrics (Gathmann and Tscharntke, 2002, Zurbuchen et al., 2010). We found no relationship between proportion of agriculture at any landscape scale and honeybee abundance, which confirmed our prediction that due to honey bee

management at many sites the landscape would not affect their distribution. Wild bee abundance and species richness were most strongly correlated with the proportion of agricultural land use at the 750 m and 1 km scales. These ranges agree with the findings of other studies correlating landscape metrics with bee abundance and diversity (Bartomeus et al., 2014), and with the predicted foraging ranges of the bees collected in this system based on body size (Gathmann and Tscharntke, 2002; Greenleaf et al., 2007).

The abundance of both wild bees and honey bees during the bloom of secondary flowers was positively correlated with yield. Although it is not surprising that the abundance of bees during the bloom of the secondaries corresponds to a higher yield of the secondary fruits, it is interesting to note that the abundance of bees during the bloom of the primaries is not correlated with the abundance of bees during the bloom of the secondaries. Consequently, we suggest that competition for pollinators with co-blooming plants could explain the difference in bee abundance between the two time points. During the primary bloom of strawberry, apple trees are also blooming, which could lead to a transient pollinator dilution for strawberry.

A positive correlation between either self- or hand-pollinated fruit weight and pollinator abundance or richness would imply that some other characteristic of the site, such as soils or microclimate, was responsible for the yield increase (as in Bartomeus et al., 2014). However, in our study, fruit weight in the self- and hand-pollinated treatments was not correlated with bee abundance, richness or agriculture in the landscape. These

results indicate that the positive correlation between bee abundance and fruit weight in the open-pollination treatment is most likely due to bee visits rather than field condition or management (see Klein et al., 2003).

While previous studies have found positive correlations between pollinator richness and fruit set or seed set (Klein et al., 2003; Hoehn et al., 2008; Fründ et al., 2013; Garibaldi et al., 2013), our data show no correlation between species richness and yield in any sampling period, despite a positive correlation between bee abundance and richness. Although species richness had little impact on yield, no farms had fewer than 10 species present. Farms with low species richness may be more vulnerable to spatial and temporal variation in yield than more species rich farms.

Given the open floral structure and self-compatibility of the strawberry flower (Zebrowska, 1998), it is likely that the number of visits is more important than the identity of the visitor (Vazquez, Morris and Jordano, 2005). Chagnon et al. (1993) found honey bees to be the most efficient pollinators of strawberry, but also proposed that complementary behaviors of honey bees and wild bees were necessary to achieve full pollination. However, our results show that high levels of pollination can be achieved even in the absence of honey bees. While Chagnon et al. (1993) grouped all wild bees together in their comparisons, our dataset allows for differentiation between genera that vary dramatically in size and behavior. Only marginal differences in pollination efficiency were found among honey bees and the four most common wild bee genera. In combination with the lack of correlation between bee richness and yield, these results

indicate a high functional redundancy in the strawberry pollinator community. On average, a single visit by a bee to a virgin strawberry flower was sufficient to increase yield by 38%. This increase is comparable to the 35% increase in average fruit weight of open-pollinated fruits compared to self-pollinated fruits in the larger landscape study, and suggests that flowers tend to be visited only once or that multiple visits to the same flower do not enhance yield.

Despite the comparable per-visit efficiency of wild bees and honey bees, the majority of pollination services provided to strawberry can be attributed to wild bees due to their greater overall abundance. The community of bees in this study was dominated by wild bees (93%) with honey bees representing only 7% of individuals collected. This finding is surprising given the relative dominance of honey bees reported in earlier studies of strawberry pollination (Chagnon et al., 1993 = 72% honey bees; Bartomeus et al., 2014 = 78% honey bees), but supports a growing body of literature demonstrating the importance of wild bees for crop pollination.

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Table 2.1. Overview of minimal adequate models describing landscape effects on pollinator abundance and richness. Models were selected using the dredge function based on second order Akaike Information Criterion (AICc).

			Bloom stage	Landscape			
Response variable	AICc	ΔAICc	Coef.	Metric (Prop.)	Scale (m)	Coef	Weight
Wild bee abundance	68.3	0	0.598	Agriculture	1000	-2.226	0.209
	69.2	0.89	-----	Agriculture	1000	-2.159	0.134
	69.9	1.59	0.599	Agriculture	750	-2.009	0.094
Wild bee richness	62.9	0	0.652	Agriculture	1000	-2.087	0.259
	64.9	1.91	0.648	Agriculture	750	-1.806	0.100
Honey bee abundance	72.4		-----	-----	-----	-----	0.07

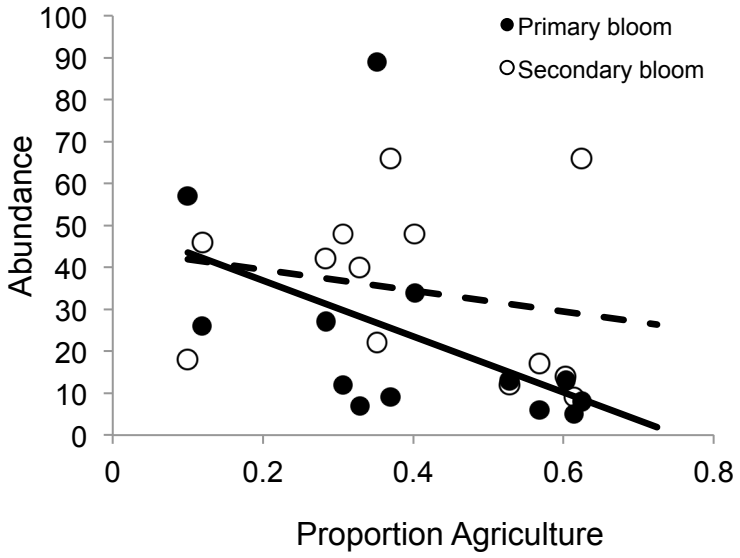

Figure 2.1. Wild bee abundance during primary bloom (continuous line) and secondary bloom (dashed line) in relation to the proportion of agricultural land within a 1 km radius around the experimental plots.

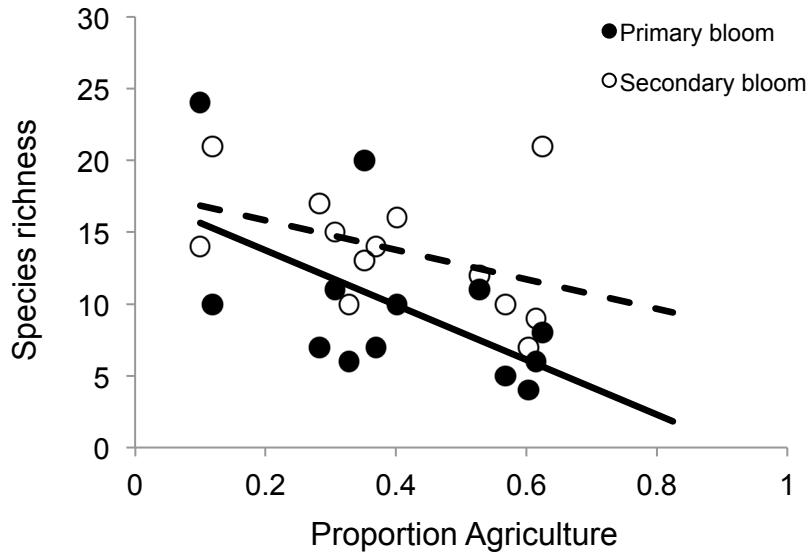

Figure 2.2. Wild bee species richness during primary bloom (continuous line) and secondary bloom (dashed line) in relation to the proportion of agricultural land within a 1 km radius around the experimental plots.

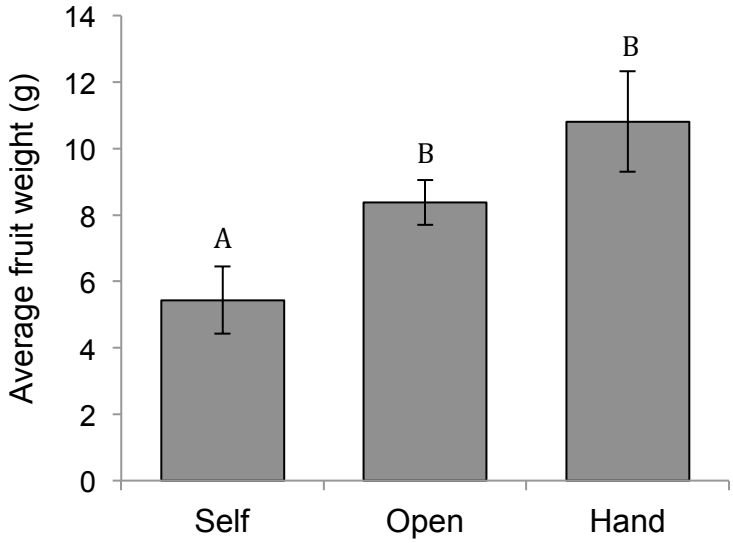

Figure 2.3. Average (+/- 1 SE) weight of self-, open-, and hand-pollinated strawberry fruits across 10 farms. Fruits in the self-pollination treatment were bagged from flowering until harvest while open- and hand-pollinated were exposed to visitors during flowering and then bagged until harvest. Hand pollinated fruits received supplementary pollen. Different letters show significant differences between treatments based on Tukey's HSD test ($p<0.05$).

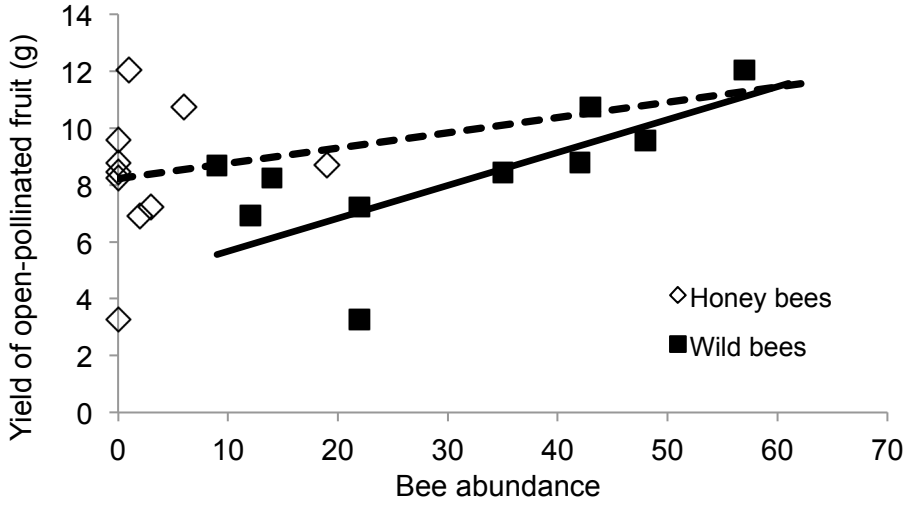

Figure 2.4. Average weight of fruits in the open-pollination treatment with respect to the abundance of wild bees (solid line) and honey bees (dashed line) during the secondary sampling period.

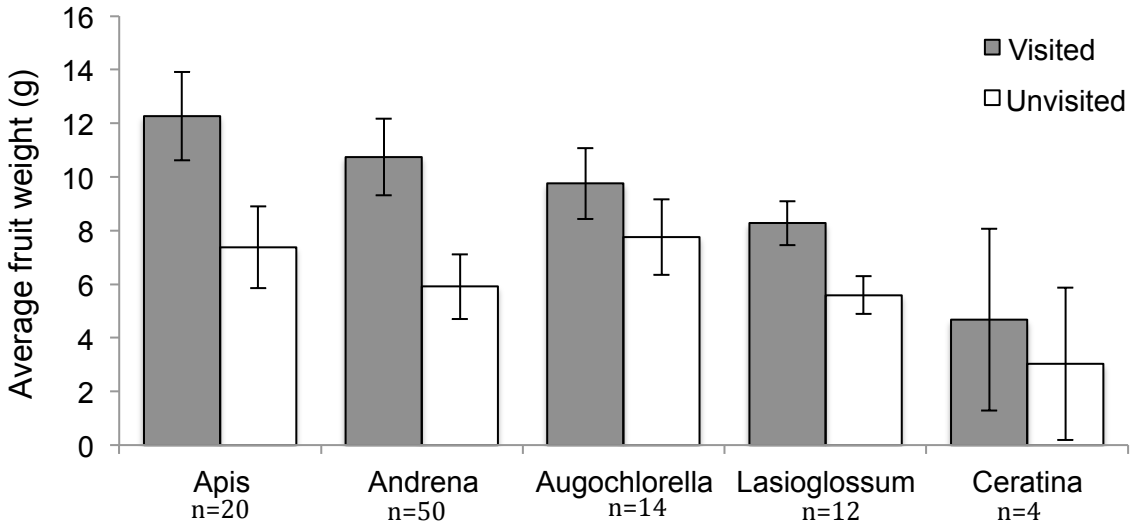

Figure 2.5. Average weight (+/- SE) of paired visited and unvisited fruits for each of the genera observed.

Table 2.S1. The complete list of bee species collected across the 14 strawberry fields surveyed in New York.

Family	Species	Abundance
Andrenidae	*Andrena algida*	1
	Andrena allegheniensis	2
	Andrena canadensis	1
	Andrena carini	6
	Andrena crataegi	5
	Andrena cressonii	13
	Andrena erigeniae	3
	Andrena erythronii	3
	Andrena icilis	1
	Andrena imitatrix	8
	Andrena melanochora	1
	Andrena miserabilis	13
	Andrena nasonii	357
	Andrena nigrihirta	1
	Andrena perplexa	2
	Andrena regularis	3
	Andrena rugosa	1
	Andrena vicina	7
Apidae	*Apis mellifera*	72
	Bombus impatiens	3
	Ceratina calcarata	25
	Ceratina dupla	8
	Ceratina mikmaqi	4
	Nomada armatella	26
	Nomada bethunei	3
	Nomada cressonii	3
	Nomada denticulata	5
	Nomada pygmaea	1
	Xylocopa virginica	1
Colletidae	*Hylaeus affinis*	4
	Hylaeus mesillae	1
Halictidiae	*Augochlorella aurata*	85
	Augochlora pura	1
	Agapostemon virescens	2
	Halictus confusus	16
	Halictus ligatus	9
	Halictus rubicundris	1
	Lasioglossum abanci	1
	Lasioglossum anomalum	1
	Lasioglossum cinctipes	5
	Lasioglossum coreaceum	7
	Lasioglossum divergens	2
	Lasioglossum ephialtum	9
	Lasioglossum foxii	3
	Lasioglossum hitchensi	150
	Lasioglossum imitatum	37
	Lasioglossum laevisimum	10
	Lasioglossum leucocomum	3
	Lasioglossum lineatulum	4
	Lasioglossum obscurum	4
	Lasioglossum paradmirandum	12

	Lasioglossum pectorale	4
	Lasioglossum perpunctatum	15
	Lasioglossum pilosum	7
	Lasioglossum versatum	79
	Lasioglossum viridatum	5
	Lasioglossum zephyrum	2
	Lasioglossum zonulum	4
	Sphecodes atlantis	3
Megachilidae	*Hoplitis simplex*	1
	Osmia bucephala	2
	Osmia inermis	2
	Osmia pumila	3
	Osmia conjuncta	1
	Osmia taurus	1
Total		1075

Figure 2.S1. Map of the study region in the Finger Lakes of New York State, USA depicting the location of farms.

CHAPTER 3

Title: Agriculturally dominated landscapes reduce bee phylogenetic diversity and pollination services

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Abstract: Agricultural intensification threatens biodiversity and has the potential to reshape the tree of life by favoring the persistence of some lineages over others. Yet it is unclear if agriculturally mediated loss of phylogenetic diversity compromises the delivery of ecosystem services upon which agricultural systems rely. To address this critical knowledge gap, we combine remotely sensed land cover data, extensive surveys of a diverse pollinator community and data on crop yield and quality with a species level bee phylogeny. Our study is the first to employ phylodiversity metrics to better understand the consequences of land use changes on critical ecosystem services. Pollinator communities in highly agricultural landscapes contain fewer evolutionary distinct taxa and 200 million fewer years of evolutionary history than more diverse landscapes. Compared to species richness, loss of pollinator phylogenetic diversity was more strongly associated with lower crop yield and quality. Policies that support the preservation of natural and semi-natural habitats on working lands will be essential for maintaining the functional and evolutionary diversity of pollinator communities and ensuring robust pollination services.

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A preponderance of evidence supports the positive relationship between biodiversity and ecosystem functioning (*1*, *2*). Yet, in communities comprised of species with high functional trait overlap, perhaps due to recent shared evolutionary history, each species has less impact on overall community function (*3*, *4*). Alternatively, communities with species drawn broadly from across the phylogeny exhibit greater stability and resilience of ecosystem services (*5*). Phylogenetic diversity is a measure the evolutionary history represented within a community. It not only captures similarities in traits that mediate responses to the landscape but also reflects similarities among taxa in the traits that contribute to ecosystem function. In order to conserve ecosystem function and associated ecosystem services, it is essential to understand the factors that impact community phylogenetic diversity and the resulting effects on ecosystem services.

Agricultural intensification of the landscape is one of the primary drivers of biodiversity loss worldwide (*7*), threatening even organisms upon which agricultural productivity relies (*8*, *9*). Bees are responsible for pollinating the majority of our most valuable and nutritious crops (*10*, *11*). Diverse bee communities comprised of many species ensures high and stable delivery of pollination services (*12*) but habitat loss and agricultural intensification have been implicated in recent bee declines (*13*). The suite of traits exhibited by different bee species mediate their ability to persist in agricultural landscapes (*14*). Thus, loss of taxonomic diversity as a result of environmental stressors is not expected to be uniform across the phylogeny (*15*) as different branches

of the tree of life may vary in their responses to land use change (*16*). However, the extent and pattern by which increasing agricultural intensification of landscapes results in pruning of the evolutionary history represented within pollinator communities and its implications for ecosystem functioning remains unresolved.

Here we quantify changes in bee phylogenetic diversity across a landscape gradient by generating a phylogeny and combining it with extensive pollinator community and fruit yield datasets. The pollinator community data are derived from sampling in 48 apple orchards over 9 years and consist of 7,500 records spanning 89 species. Landscape composition in a 1km radius surrounding each orchard varied from mixed forest, urban, old-field and agricultural to landscapes dominated by agricultural land covers. Fruits of the two most common apple cultivars (Golden Delicious and McIntosh) were harvested from 17 of the surveyed orchards and the weight of each mature fruit was recorded along with the number of seeds and a quality score indicating the symmetry of the fruit. Our analysis focused on three unresolved questions. First, how does agricultural intensification at the landscape scale influence the phylogenetic structure of pollinator communities in agro-ecosystems? Second, are evolutionarily distinct taxa at greater risk from agricultural intensification? And lastly, what are the consequences of phylogenetic diversity loss on ecosystem services such as pollination and crop production?

We found that, while individual taxa varied in their response to increasing agricultural land cover, closely related species tended to have more similar responses than species pairs selected at random. (Bloomberg's K = 0.72, P = 0.043; Figure 1). The clades most

resilient to land use change included the family Halictidae and the *Andrena* clade comprising the subgenera *Andrena*, *Plastandrena* and *Trachandrena*. Clades that are most resilient to increasingly agricultural landscapes possess many of the traits previously associated with persistence in these habitats: small body size, broad host-plant range, and ground nesting habit (*17*–*22*). However, our analyses reveal several clades sensitive to land use change that, based on traits alone, would have been predicted to be tolerant including the *Andrena* subgenera *Simandrena* and *Melandrena,* which were identified as the most sensitive groups. These differences likely stem from an incomplete understanding of the traits that mediate responses to land use change or reflect the difficulty in measuring particular traits such as pesticide sensitivity or pathogen resistance across a broad range of taxa.

Our analyses reveal that species loss due to changes in the composition of agricultural landscapes is not random across the bee phylogeny. Rather, some branches of the bee tree of life are “pruned” more heavily than others resulting in communities that contain more closely related species compared to those found in landscapes with lower agricultural landcover ($F_{(1,45)}$ = 15.47, P < 0.0001; Figure 2a). Although species richness was 39% lower at orchards with a high proportion of agriculture in the landscape ($F_{(1,45)}$ = 30.14, P < 0.0001; Figure 2b), the effect on PD remained even after accounting for loss in species richness. For every 10% increase in the agricultural cover within the landscape, 48 million years of evolutionary history is lost from pollinator communities ($F_{(1,51)}$ = 8.61, P = 0.005; Figure 2c).

Evolutionarily distinct taxa, those with few close relatives (ie. long branches),are likely to have traits that are dissimilar compared to closely related taxa. Loss of evolutionarily distinct taxa has implications for the shape of the future tree of life and represents a significant loss of evolutionary potential and, consequently, reduced ability to respond to future disturbances. Here, we find evidence for loss of evolutionarily distinct taxa spread broadly across the bee phylogeny with communities in highly agricultural landscapes having 36% lower average distinctiveness ($F_{(1,50)}$ = 4.62, P = 0.036; Figure 2d). Recent studies within the genus *Bombus* find that distinct lineages are not more likely to have received International Union for Conservation of Nature (IUCN) red list status (*23*). In light of these findings, our results suggest that extirpation of distinct taxa may occur locally without substantially increasing the risk of extinction of evolutionarily distinct lineages.

Closely related bee species may share behavioral and morphological traits that are important for pollination including body size, plant fidelity, pollen carrying habit, visitation rate and behavior on flowers. After accounting for the effects of species richness, sites with greater phylogenetic diversity had greater fruit weight ($F_{(1,333)}$ = 24.22, P < 0.001; Figure 3a), greater number of seeds per fruit ($F_{(1,333)}$ = 17.33, P < 0.001; Figure3b) and more symmetrical fruit ($F_{(1,333)}$ = 17.71, P < 0.001; Figure3c) but only for Golden Delicious and not for McIntosh (Figure 3d-e). Re-analysis without including honey bees produces similar results (see SI). These results suggest that introducing managed pollinators will not compensate for observed losses in phylogenetic diversity due to agricultural intensification.

Our results reveal that landscape scale agricultural intensification contributes to the loss of evolutionary history from bee communities and has consequences for current ecosystem functioning. In light of ongoing increases in agricultural intensification worldwide (*24*), our results have implications for both the functional and evolutionary potential of bee communities to respond to future challenges. Conservation practices that measure their success only by the number of species conserved may fail to protect the full diversity of life impacted by agricultural intensification (*25*). Greater understanding of how shared evolutionary history shapes responses to current stressors is essential for assessing the potential mechanisms driving bee declines in agricultural landscapes and in informing conservation efforts. Conservation practices that promote a functionally and evolutionarily diverse set of taxa, such as the preservation of natural and semi-natural habitats on working lands, will be essential for maintaining diverse pollinator communities and robust pollination services into the future.

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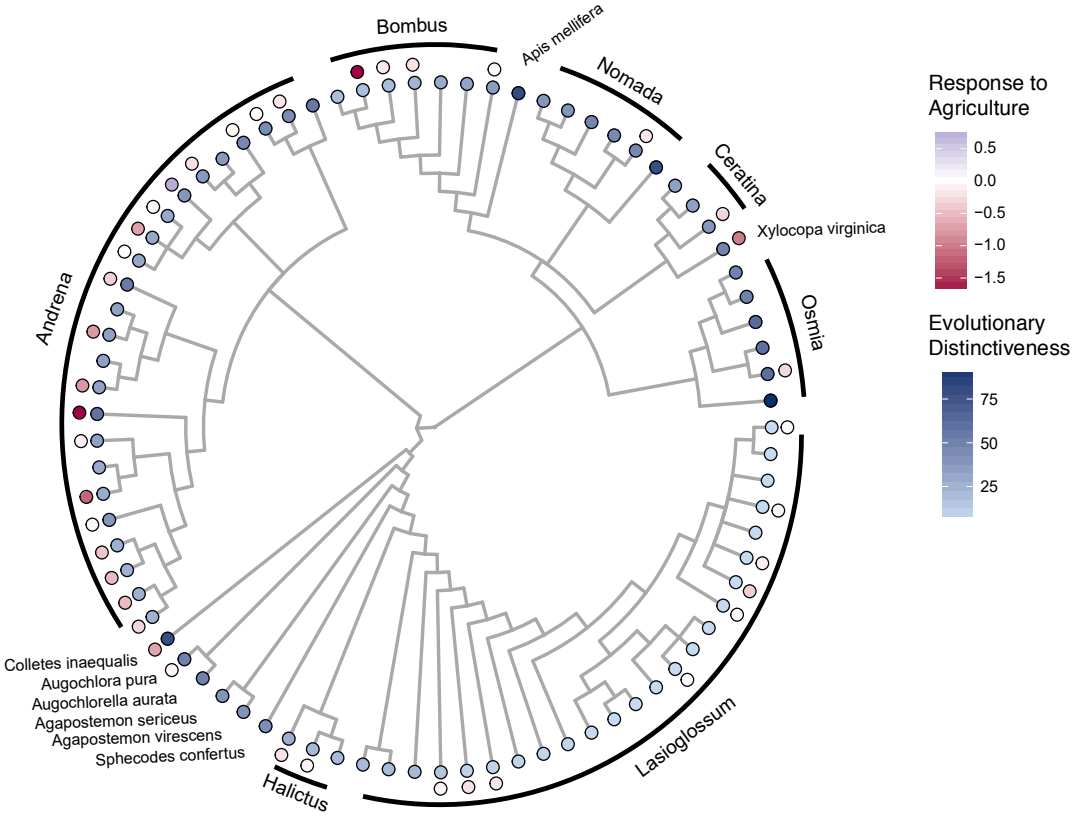

Figure 3.1. Time calibrated phylogeny of the apple bee community. First tip labels indicate evolutionary distinctiveness of taxa measured by the fair proportion metric. Second tip label only shown for the 41 taxa observed greater than 10 times across all surveys with tip symbols representing their response to increasing agricultural land cover at the 1km scale.

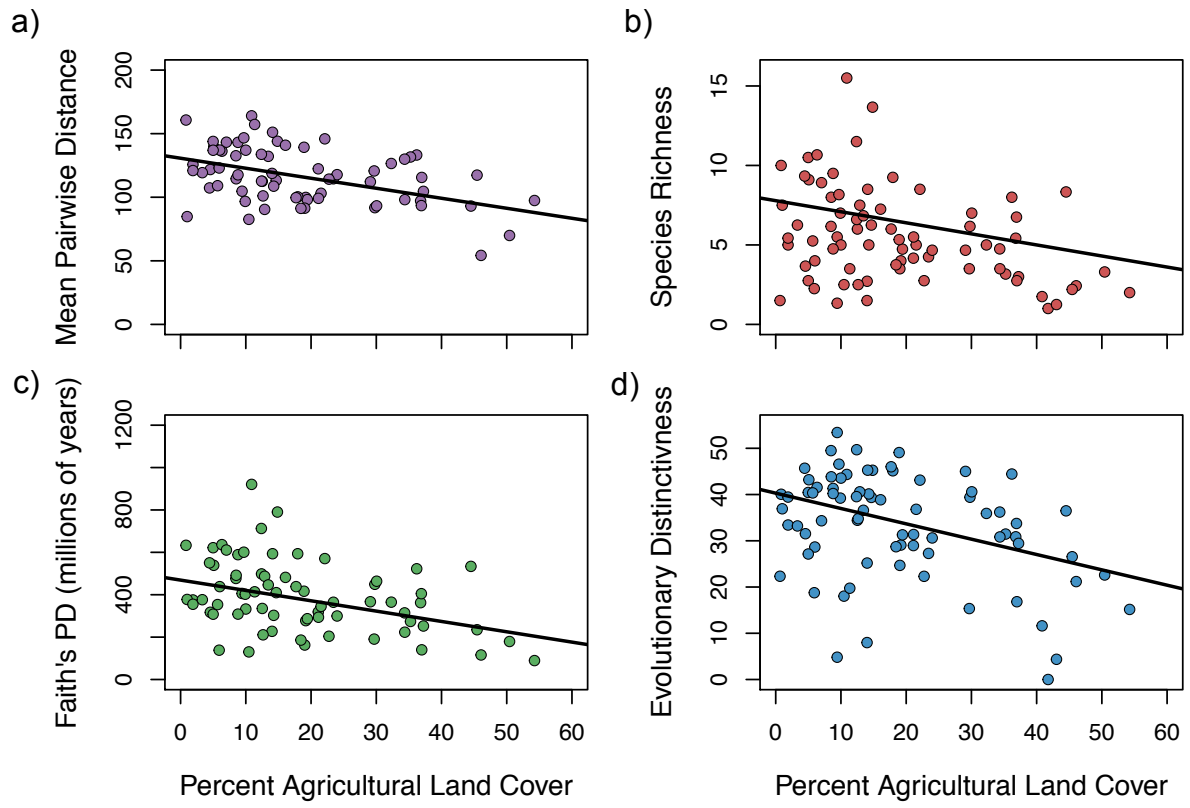

Figure 3.2. Relationship between agricultural cover in the surrounding landscape at a 1km radius and community diversity metrics. a) Individuals are more closely related to one another in high agriculture landscapes, b) fewer species are observed per transect c) the evolutionary history represented by communities is lower and d) the average evolutionary distinctiveness of taxa is lower.

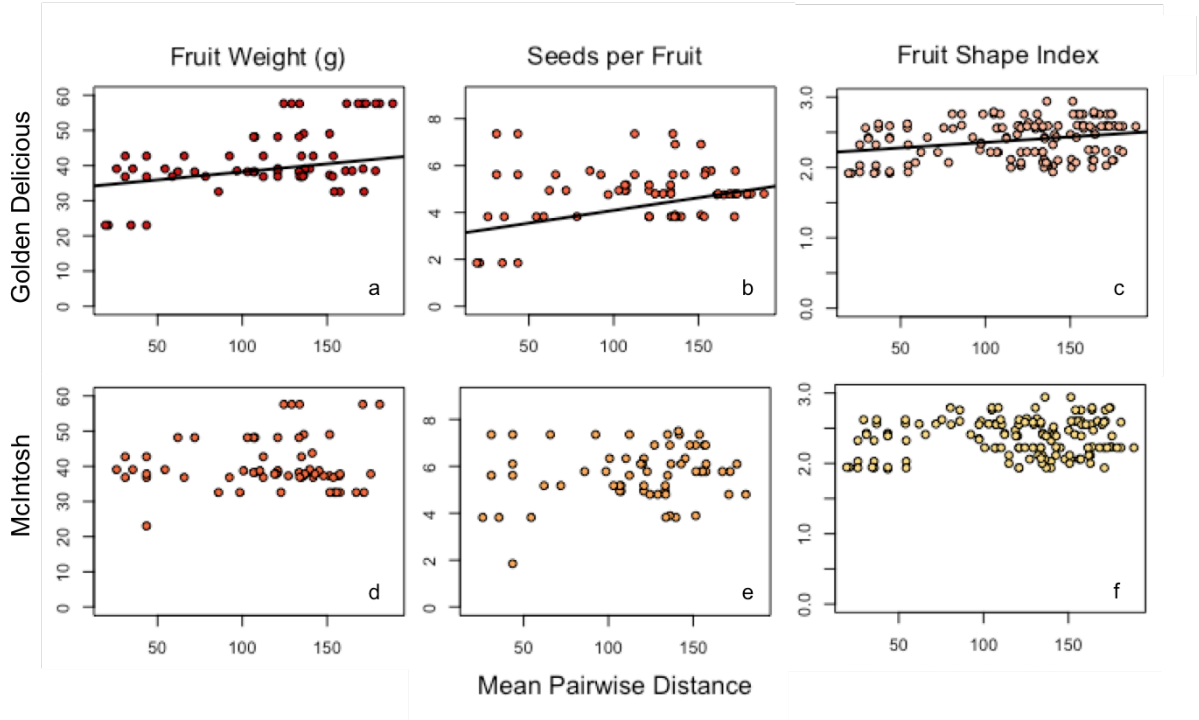

Figure 3. Relationship between measures of apples yield (a-b, d-e) and quality (c,f) and Mean Pairwise Phylogenetic Distance separating individuals in a community. Regression lines indicate a significant relationship between variables at $P < 0.05$.

Supplementary Info

Methods:

Bee collections

In order to determine the effect of landscape composition on the phylogenetic structure of pollinator communities, we used a long-term dataset of bee community censuses in eastern apple (*Malus domestica*) orchards. The data span 7 years (2008-2014) over 27 sites in New York State, the second largest apple producing state in the US (USDA NASS 2015). At each site, bees were collected while foraging on apple blossoms during timed 15 min transects covering 100m of orchard row length. The full dataset includes 7,500 records from 456 transects representing 115 collecting hours. Specimens were identified to species using published revisions (*1–5*) and reference materials maintained in the Cornell University Insect Collection (http://cuic.entomology.cornell.edu/). The pollinator fauna in this dataset and sampling protocols are described in more detail in Russo et al (*6*) and Park et al (*7*).

Phylogeny

DNA was extracted from recently collected pinned specimens of 81 of the 89 observed species following standard protocols detailed in Danforth (*8*). The remaining 8 species were recorded fewer than 3 times and recent specimens for DNA extraction could not be obtained. However the absence of these species from the analysis is expected to have little impact on the results as they are distributed widely across the phylogeny. Furthermore, the 81 taxa included in the analysis represent 91% coverage of the species richness and 99.3% coverage of all individuals recorded. One nuclear

ribosomal gene (28S), one mitochondrial gene (COI) and one nuclear protein-coding gene (EF1-a) were amplified for each taxon following standard PCR methods. PCR products were purified using ExoSAP-IT (Affymetrix). All PCR products were sequenced in both directions on an ABI 3730x1 capillary DNA Analyzer using Big Dye Terminator chemistry.

Sequences were edited and aligned using the G-INS-i algorithm implemented in MAAFT v7 (*9*). The final 2505 nucleotide alignment was divided into partitions using PartitionFinderV1.1.1 (*10*) resulting in 11 subsets, which were then analyzed in MrBayesv3.2.6 (*11*, *12*) accessed through the CIPRES portal (*13*). The analysis consisted of 2 runs with 4 chains each for 10 million generation sampling every 2000. The first 25% of the run was discarded as burnin before a maximum clade credibility tree was constructed. The consensus tree was transformed to be ultrametric and time-calibrated based on estimated family divergences from Cardinal & Danforth (*14*) based on penalized likelihood using a relaxed clock model for substitution rate variation among branches and a lambda rate smoothing parameter of 0 estimated from the data (*15*).

Landscape analysis

Landscape composition surrounding each of the 27 study sites was characterized within a 1 km radius using the National Agricultural Statistics Service New York State Cropland Data Layer for the year corresponding to the year of sampling in ArcGIS 10.1. The 1 km scale was chosen as it was found to be the best predictor of bee abundance and diversity in this system (*7*). Using these maps we estimated the cover of

agricultural land uses (all crop categories including forage and pasture) as well as natural and semi-natural habitats (forest, wetlands, shrub lands, meadows, and fallow).

Apple Harvest Data

To evaluate the potential for loss of phylogenetic diversity to negatively impact pollination services we used data on apple weight and quality that was available from 17 of the surveyed orchards for both Golden Delicious and McIntosh cultivars. These two cultivars are the most commonly grown across out study region yet, the flower morphology of the cultivars differs two ways that make McIntosh less sensitive to visitor diversity. First, the positioning of stamen filaments in McIntosh reduces the diversity of behaviors exhibited by floral visitors (*16*, *17*). Secondly, the flowers of McIntosh are perfectly syncarpic such that pollen deposition on only one of it's five fused stigmas can still result in full compliment of ten seeds being produced (*18*).

All fruits from one branch on six trees of each cultivar per site were harvested when mature. Selected branches were of approximately the same diameter, length and position on the tree to minimize horticultural effects. The weight of each mature fruit was recorded along with the number of seeds and a quality score indicating the symmetry of the fruit which ranged from 1: badly misshapen and unmarketable, to 3: perfectly symmetrical fresh market quality.

Statistical methods

Faith's Phylogenetic Diversity (PD), the distance on the phylogeny separating members of a local community, was calculated as the summed branch lengths of our time calibrated ultrametric phylogeny for all species observed in a given transect. Although PD can provide direct insights into the total evolutionary history present in a given community, it is often correlated with species richness (SR). Therefore, we calculated the abundance weighted Mean Pairwise Distance (MPD), which measures the average distance separating any two individuals in a community and is less influenced by species richness (REF). Additionally, we used the phylogeny to quantify the Evolutionary Distinctiveness (ED) for each species using the Fair Proportion metric, which partitions branch lengths for each species by the total number of species subtending it (REF), and calculated the average distinctiveness of taxa from each transect.

To determine whether changes in landscape composition influenced each diversity metric, we used generalized linear mixed effects models with Poisson error structures for count data (SR) and Gaussian errors for continuous data (PD, MPD, ED). Fixed effects included either agricultural or natural land cover land cover and random effects included year and site as nested effects. Statistics are reported for the most predictive land cover type (See SI for alternative models); although there is a strong negative correlation between agricultural and natural land cover (STATS). For these analyses we calculated each metric (SR, PD, MPD, ED) on the local communities excluding honey bees. The presence of honey bees and their abundances likely reflect pollinator management practices within the orchards rather than landscape composition in the surrounding area.

We evaluated the response of each species that was recorded more than 10 times across the survey period to agricultural land cover in separate generalized linear mixed effects models. All models used Poisson error structures and included the nested random effects of year and site. We then estimated the phylogenetic signal for response to agriculture, measured as the strength of the co-variation in the coefficients of the response to agriculture, among taxa with respect to the variation in phylogenetic distances between them. We used the Bloomberg's K statistic, which compares the observed signal in a trait to the signal under a Brownian motion model of trait evolution (*19*). Significance was assessed by comparing the observed value to variance of phylogenetically independent contrasts relative to a tip shuffling on 999 null models.

To determine whether changes in SR and MPD (including honey bees) resulted in changes in the delivery of pollination services, we used generalized linear mixed effects models with a Gaussian error structure on the averaged fruit metrics for each site. Random effects in each model included year nested within site.

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CHAPTER 4

Title: Temporally dependent pollinator competition and facilitation with mass flowering crops affects yield in co-blooming crops

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Abstract

One of the greatest challenges in sustainable agricultural production is managing ecosystem services, such as pollination, in ways that maximize crop yields. Most efforts to increase services by wild pollinators focus on management of natural habitats surrounding farms or non-crop habitats within farms. However, mass flowering crops create resource pulses that may be important determinants of pollinator dynamics. Mass bloom attracts pollinators and it is unclear how this affects the pollination and yields of other co-blooming crops. We investigated the effects of mass flowering apple on the pollinator community and yield of co-blooming strawberry on farms spanning a gradient in cover of apple orchards in the landscape. The effect of mass flowering apple on strawberry was dependent on the stage of apple bloom. During early and peak apple bloom, pollinator abundance and yield were reduced in landscapes with high cover of apple orchards. Following peak apple bloom, pollinator abundance was greater on farms with high apple cover and corresponded with increased yields on these farms. Spatial and temporal overlap between mass flowering and co-blooming crops alters the

strength and direction of these dynamics and suggests that yields can be optimized by designing agricultural systems that avoid competition while maximizing facilitation.

Introduction

Increasing consumption driven by a growing global population and demands for more varied and resource intensive diets has placed unparalleled strain on our agricultural production systems and natural resources. Current agricultural practices rely on fossil fuels, agrochemicals and conversion of new agricultural lands. Yet, yield gains produced through these practices have plateaued in recent years [1] and have come at the cost of increasing greenhouse gas emissions, degradation of water quality, widespread pollution, pesticide resistance and unprecedented biodiversity loss. An alternative solution to meet the planet's growing needs is ecological intensification, increasing production on existing farmlands in ways that causes less harm on the environment through the replacement of anthropogenic inputs with ecosystem services management [2,3]. Manipulating and regulating the biotic interactions underpinning the provisioning of ecosystem services by increasing the structural diversity of agroecosystems had been demonstrated to improve crop yields [4–6]. In order to implement diversification strategies successfully, it is critical to understand whether agricultural habitats themselves may act as sources of ecosystem services or whether diversification may lead to competition for services among crops. Certain crops may have a disproportionate effect on the flow of ecosystem services due to the large pulse of resources they provide[7,8], and it is essential to understand the effects of these crops

on ecosystem service dynamics in order to develop effective management strategies that can be directly implemented by land managers.

Crops that are grown on large scales and bloom *en masse* create large pulses of resources that have substantial impacts on communities of ecosystem service providers. These dynamics are particularly relevant for pollinator dependent crops given the dramatic increase in the area planted to these crops [9] and their importance for human nutrition [10,11]. Pulses in floral resources associated with mass blooming of crops are known to alter pollinator abundances and visitation rates in nearby crops and natural habitats [12–15], which are likely to have direct impacts on crop yields [16]. Mass flowering crops can increase pollinator offspring production [17] and pollinator densities following mass bloom [18,19], particularly for solitary, univoltine bees [20] for which the bloom of a single crop may represent nearly the entire span of their adult foraging activity. Therefore, mass flowering crops may facilitate pollination of co-blooming crops when pollinators attracted and supported by the mass blooming crop spill over into the co-blooming crop, augmenting floral visitation and crop pollination [21] (Figure 1). Alternatively, when pollinators are limited, as is common in simplified agricultural systems [22], plants with high overlap in their pollinator community may compete for visits from shared pollinators [23] (Figure 1).

Little is known about competitive or facilitative interactions between pollinator dependent crops with respect to pollinators and pollinator services; however, we expect that these interactions are ubiquitous in agricultural landscapes. They only require that crops have overlap in their pollinator community though they may bloom in different seasons [21] or even in different years [20]. The likelihood of the interaction resulting in

either facilitation or competition depends not only on the degree of overlap in the pollinator community [24] but also on the temporal overlap in bloom between crops [13,21,25]. Indeed, the two studies available on the effects of mass flowering crops on wild plants have found that mass flowering crops can either reduce [26] or enhance pollination [13] in co-blooming plants in nearby natural habitats.

At the landscape scale, greater abundance and diversity of bees associated with natural and semi-natural habitats [22] may buffer against local competition or facilitation effects. For example, in landscapes with high amounts of natural habitat, competition between co-blooming crops may be lower than expected [14]. In this case, although bees are drawn to the mass blooming crop, the number of bees moving from natural habitats into the co-blooming crop may still be sufficient to provide adequate pollination services[7]. Alternatively, proximity to natural habitats may reduce facilitation when bees move from mass blooming crops to alternative forage in natural habitats rather than the co-blooming crop.

Despite the potential importance of pulsed resource dynamics for crop pollination and associated yield, we are not aware of any studies that have evaluated the effects of mass flowering crops on the yield of another crop. Greater understanding of spatial and temporal factors that shift the balance between competition and facilitation will allow for management practices that maximize crop yields under the pulsed resource dynamics characteristic of agroecosystems.

In this study we investigate the potential for pollinator mediated competition or facilitation in two economically important crops: apple (*Malus domestica*), a mass flowering crop, and strawberry (*Fragaria x ananassa* Duch.) in central New York, USA.

In this region apple and strawberry have a staggered but overlapping bloom period. The two crops are both members of the family Rosacea, and thus expected to have high overlap in their pollinator faunas [27,28]. Furthermore, the community of bees visiting both apple and strawberry is dominated by early spring, ground-nesting, univoltine bees in the genus *Andrena* [29–31]. The high potential for community overlap in pollinators between apple and strawberry make these two crops an ideal study system in which to understand the potential for pollinator-mediated interactions among crops.

We hypothesized that the impact of apple on strawberry pollination may vary temporally, with facilitation and competition occurring in the same system but at different stages of apple bloom. Additionally, we hypothesized that proximity to natural habitats would moderate these effects and predicted that sites in close proximity to natural habitat would have greater bee abundance and experience both reduced competitive and facilitative effects.

Methods

Both apple and strawberry are economically important crops in the United States, with total apple production at 327,000 acres and strawberry production at 61,000 acres (USDA NASS, 2013). In New York State, the second largest apple-producing region in the US (USDA NASS, 2013), it is common for farms to grow apples plus a range of other small fruit crops including strawberry.

Study Sites

Studies were carried out in the spring of 2013 in the Finger Lakes Region (42°26'N, 76°30'W) of New York, USA. The study area is characterized by a diversity of agricultural uses, including dairy, row crop, tree fruits and vegetables with natural and semi-natural areas of deciduous forest, small woodlots and old field dispersed throughout. A total of 35 farms growing apple, strawberry or both were identified. All farms were used to estimate pollinator community similarity and a subset of 13 farms, all growing strawberry but with a gradient in the proportion of apple orchard cover in the surrounding landscape (0 - 37%), were used in further experiments. Focal strawberry fields on each of these 13 farms were selected. The landscape surrounding the focal strawberry field was characterized within a 1 km radius using the 2013 National Agricultural Statistics Service Cropland Data Layer for New York [32] in ArcGIS 10.1. Using these maps we estimated the proportion of land in agricultural uses (all crop categories including forage and pasture), natural and semi-natural areas (forest, wetlands, shrub lands, meadows, and fallow), and apple orchards. Apple orchards flowered between May 3 and June 5, 2013, with bloom initiation and duration varying across farms depending on local microclimate and apple variety. Most farms cultivate a number of apple varieties to ensure pollination success, as apple is varietally self-incompatible. Measurements occurred between May 6 and May 9 for early apple bloom, between May 14 and May 17 for peak apple bloom and between May 31 and June 3 for late apple bloom. Apple flowering phenology in 2013 would be described as “typical” based on historical data on apple flowering in upstate NY [33]. In the early stage of apple bloom the percentage flowers open of total flowers present, estimated by counting the number of open flowers per cluster on randomly selected trees, averaged 26.6% (± 5.4

SE). During the same period, strawberry bloom had initiated only at four sites (with 16.9 % ± 11.5 SE flowers open). At peak apple bloom, flowering intensity averaged 54.8% (± 5.8 SE) compared to strawberry bloom at 23.2 % (± 7.3 SE). Apple bloom intensity during the late flowering stage averaged only 10.9% (± 3.5 SE) while strawberry bloom was 37.5 % (± 6.7 SE).

We quantified apple mass flowering using a mass flowering index. The index describes the total amount of apple flowering within the surrounding landscape and is calculated as the percent apple cover in a 1 km radius around the focal strawberry field multiplied by the percent of open apple flowers in adjacent orchards (if present). Thus, the highest values of the mass flowering index indicate high abundance of apple flowers locally and within the landscape.

Pollinator Community

To estimate similarity in the apple and strawberry pollinator communities, bees were collected using sweep netting during four 15-minute surveys along 100m transects in apple orchards and strawberry fields during the peak bloom of each crop. Surveys were conducted between 10:00 and 15:30 on sunny days with temperature above 16°C. Bees were identified to species using published revisions [34–39] and online keys (Discoverlife.org) as well as expertly identified reference materials maintained in the Cornell University Insect Collection (http://cuic.entomology.cornell.edu/).

In order to understand how mass flowering apple impacts bee visitation to strawberry, we estimated the abundance and diversity of bees visiting strawberries over the course of the apple bloom within each focal strawberry field and adjacent to the

nearest natural or semi-natural habitats. Distances between strawberry fields and semi-natural habitats on a farm ranged from 20 to 300 m (mean = 46 ± 9 m). Arrays of four white pan traps were placed at 3 m intervals on transects 2 m from the edge of each focal strawberry field and semi-natural habitat during three sampling periods corresponding to the early, peak and late stages of apple bloom. White pan traps were used as they collect a greater number of bees but maintain a similar community composition to sweep net sampling compared to other trap colors (H. Grab, *unpublished data*). Sampling periods were approximately one week apart, varying based on local microclimatic conditions, beginning on May 6th and ending June 3rd 2013. The intensity of strawberry and apple bloom was recorded when the arrays were set out and when they were collected. Bloom intensity was estimated as the percentage flowers open of total flowers present including senesced flowers and flowers in bud stage in the orchards or fields. These data were then averaged in order to estimate bloom intensity during each stage. Pan traps were collected after 72 hours and the contents were sorted and identified to species. Sampling rarefaction curves for species richness are available in the Supplementary Information (Figures S1-3).

Strawberry pollination

To assess the effects of apple mass flowering at the landscape scale on strawberry pollination and fruit set, we measured the pollination rates of sentinel strawberry plants placed within the focal strawberry field and adjacent to semi-natural habitat at each farm. Use of sentinel plants allowed us to maintain consistent strawberry bloom density during each stage of apple bloom and to control for abiotic factors,

including soil and microclimate that could affect yield. During the three periods corresponding to the early, peak and late stages of apple bloom, we placed 10 individually potted strawberry plants (variety Evie 2) in the same transects used for pollinator sampling described above. Strawberry plants have one primary flower, two secondary flowers and up to four tertiary flowers per inflorescence. The number of achenes is greatest on primary fruit and decreases in subsequent flowers. Only primary and secondary fruits were used to estimate yield, as they are the only fruits usually considered marketable. These flowers were exposed to visitors for 72 hours but on half of the plants, flowers received supplementary pollen applied with a paintbrush to the stigmas. These hand-pollinated fruits, when compared to open-pollinated fruits, allowed us to estimate the relative contribution of the pollinator community to yield while controlling for environmental factors such as microclimate, which may have varied slightly across the study region. We collected the sentinel plants after 72 hours and maintained them in a greenhouse chamber while the fruits developed. Fruits were harvested daily when ripe and weighed. In strawberries, fruit weight can provide an accurate estimate of pollination rate, as strawberries are an aggregate accessory fruit comprised of as many as 300 individual achenes [40]. Each achene must be fertilized in order for the surrounding tissue to develop. Hence, the weight of a fruit is highly correlated with the number of pollinated achenes [41] and an average of four pollinator visits per flower is required to achieve full pollination and maximum fruit weight [42]. Only fruits with a high percentage of fertilized achenes will develop without major malformations that reduce overall yield and marketability.

Statistical Analyses

The effect of apple mass flowering on bees was assessed using mixed effects models in the R package "nlme" [43] with either the dependent variable of bee abundance (total number of bees collected during a sampling stage at each site) or bee diversity (number of bee species collected during a sampling stage at each site) in separate models. In both models the fixed effects included natural habitat proximity (adjacent or distant from nearest natural area), apple mass flowering index, the percentage of strawberry bloom, apple bloom stage (early, peak, or late), and all possible interactions between the fixed effects. Mass flowering index was log(x+1) transformed to meet distributional assumptions. Farm was included as a random effect in the model describing bee abundance. In the final model describing bee diversity, natural habitat proximity nested within farm was included as a random effect because proximity was removed as a fixed effect following model simplification.

We used linear mixed effects models to assess the relationship between bee abundance and diversity and the average weight of strawberry fruits. Models were fit separately for bee abundance and diversity as fixed effects along with pollination treatment, apple bloom stage and location. Following model simplification the final models retained only the main effects of abundance or diversity. To account for non-independence of samples and the nested experimental design structure, random effects in the final model included the nested effects of stage within pollination treatment within the natural habitat proximity variable within farm.

In order to determine the indirect effects of the apple mass flowering index on strawberry fruit weight during each of the apple bloom stages, we used separate mixed

effects models with fruit weight as the response variable. The predictor variables included pollination treatment, the mass flowering index, and all possible interactions between the fixed effects. Fruit order (primary or secondary) nested within the natural habitat proximity variable nested within farm was included as a random effect in each model to account for the nested sampling design. In the model describing the effects during peak bloom, weight was log transformed to meet distributional normality assumptions.

All models were computed in R v. 3.2.3 [44]. Minimum adequate models were selected using backwards-stepwise selection, eliminating predictor variable with p values < 0.1. Once minimum adequate models were identified, the anova function was used to assess significance of each factor by obtaining *F* and *p* values. In all models apple mass flowering index values were $\log_{10}(x+1)$ transformed to account for overdispersion due to some farms having very high percentages of apple cover.

Results

Community Similarity. Using bees collected in sweep-net transects in apple orchards (*n* = 18 orchards, abundance = 776, species = 51) and strawberry fields (*n* = 17 fields, abundance = 994, species = 60) during peak bloom of each crop, we compared the overlap in pollinator communities of each crop. We found that apple and strawberry share 31 of the 79 pollinator species collected including the most abundant pollinators of each (Figure 2). In this region, honey bees, *Apis mellifera*, are often brought into orchards for apple pollination but not for strawberry. These managed honey bee colonies are moved out of apple orchards following peak apple bloom; therefore, we

present honey bee abundance separately from the wild pollinator community. In apple orchards, honey bees comprised 48% of the pollinator community; while in strawberry, honey bees comprised only 1.3% of the bees collected. Because our estimates of community overlap are based on collections from geographically separated locations, they represent a conservative measure of the overlap in apple and strawberry pollinators that is likely to occur within a farm.

Bee Response to Mass Flowering Apple. There was a significant effect of apple mass flowering on the abundance and diversity of bees collected in strawberry fields and adjacent semi-natural habitats that was dependent on the bloom stage (Table 1 and Figure 3). When further exploring the interaction between stage and the mass flowering index (Table 1) we found that abundance and diversity of bees collected near the sentinel plants were negatively affected by mass flowering during both early and peak apple bloom and positively affected by mass flowering during late apple bloom (Figure 3, Table S1). Bee community composition was marginally effected by the stage of apple bloom (Figure S4). As expected, bee abundance was marginally higher adjacent to semi-natural areas (mean = 16.14, SE = 2.47) compared to strawberry fields with no adjacent semi-natural habitats (mean = 9.01, SE = 2.21 Table 1). However, natural habitat proximity did not interact with either stage or the mass flowering index suggesting that the proximity to natural habitat did not alter the impact of mass flowering on the pollinator community. Although species richness was not different between strawberry fields and natural habitats, the composition of bee communities differed

between locations (Figure S5). The local intensity of strawberry bloom did not impact bee abundance or diversity at any stage, and was therefore removed from all models.

Strawberry Yield. The average weight of strawberry fruits from sentinel plants increased with both greater bee abundance ($F_{1,13}$=5.72 p=0.03) and diversity ($F_{1,13}$=24.22 p=<0.001) (Figure 4). Although pollinator abundances were greater near to natural habitats, fruit yield did not vary with natural habitat proximity. Similar to the effects observed on the pollinator community, we found the effects of apple mass flowering on strawberry fruit weight differed with the stage of apple bloom (Table 2). During both early and peak apple bloom, an interaction between pollination treatment and mass flowering impacted strawberry fruit weight (Table 2). In accordance with the competition hypothesis, mass flowering of apple decreased the weight of open pollinated strawberry fruits but not hand pollinated fruits (Figure 5, Table S2). Conversely, during late apple bloom the mass flowering index was positively associated with fruit weight (Figure 5, Table S2) suggesting facilitation during this stage.

Discussion

Resource pulses are a common feature of agricultural systems; however, the impact of mass flowering crops on the pollination and yield of co-blooming crops is currently unknown. Here we examined the spatial and temporal effects of a mass flowering crop on bee communities and subsequently on yield in a co-blooming crop species. We predicted that changes in pollinator abundance over the course of mass flowering would lead to either competition or facilitation at different stages, and indeed

we found that apple mass flowering first decreased strawberry pollination and then increased strawberry pollination with corresponding effects on yield.

The mass flowering of apple negatively affected bee abundance and diversity in co-blooming strawberry during the early and peak stage of apple bloom. However, during the late bloom stage, increasing apple mass flowering was associated with greater bee abundance and diversity in strawberry. These results indicate that bees are responding to local changes in resource availability resulting in a dilution of bees when floral resources are plentiful during early and peak apple bloom followed by a spillover of bees from apple orchards to nearby strawberry fields as apple flowering decreases. In natural systems, similar effects have been observed in mixtures of flowering *Cirsium* and *Raphanus* plants where the balance between pollinator mediated competition and facilitation was dependent on the relative densities of *Cirsium* flowers [45]. These patterns may be explained by changes in the foraging preferences of the pollinator community, but population level responses to floral resources pulses may support overall greater abundances of pollinators in landscapes with high cover of mass blooming crops[18,19,21]. Our findings indicate that both density and timing of flowering are important predictors of the outcome of these interactions.

We predicted that both abundance and diversity of bees would be greater at sites adjacent to natural habitats. Although bee abundance was greater at sites adjacent to natural habitats, bee species richness did not differ between sampling locations. This result is likely due to a greater density of nesting sites in less disturbed natural areas for the ground-nesting bees that dominated the pollinator community [46]. While the distance between strawberry fields and semi-natural habitats within a farm was not greater than

the flight distance of the average strawberry pollinator [47], it is possible that fewer individuals traveled that distance. Despite overall greater pollinator abundances in sites adjacent to natural habitats, lack of a significant interaction between the natural habitat proximity variable and mass flowering indicated that proximity to natural habitat did not alter the impact of mass flowering on the pollinator community. Furthermore, the influence of mass flowering apple on the abundance and diversity of bees was greater than the influence of proximity to natural habitats. Similar results were reported by Westphal et al. [18], who found that bumble bee densities were positively related to the availability of oilseed rape and not natural habitats within the landscape. Our findings reveal that these effects extend to a much broader pollinator community. These findings also suggest that the effects of agricultural habitats on pollinator communities has thus far been underestimated and likely represents a common phenomenon among crops with overlapping pollinator communities.

Mass flowering of apple at the landscape scale was negatively associated with the weight of open pollinated strawberry fruits during early and peak apple bloom and positively associated with fruit weight during late apple bloom. We hypothesize that these results are due to the parallel changes observed in the abundance and diversity of pollinators, as both measures were highly correlated with the weight of open pollinated strawberry fruit; however, the decrease in fruit weight associated with early and peak apple bloom may also be due in part to increased rates of heterospecific pollen transfer [48] from apple to strawberry. In the late sampling period, the positive response of hand-pollinated fruit to the mass flowering index may have been caused by

incomplete effectiveness of the hand pollination treatment due to the greater storage time of the supplemental pollen at this stage.

The competitive interactions observed between apple and strawberry likely represent a conservative estimate of the potential magnitude of indirect interactions mediated by shared pollinators. In this case, competitive effects are moderated by the relatively diverse pollinator community of strawberry [49] and the ability of strawberry to self-pollinate [42]. Therefore, the negative effects of mass flowering may be stronger in crops that are more pollinator dependent or share a greater proportion of their pollinator community with a mass flowering crop.

In natural systems, pollinator-mediated facilitation in plant communities is thought to occur through several mechanisms. First, coexisting plants may attract greater numbers of shared pollinators by providing aggregate floral displays greater than a single species alone [45]. Facilitation may also occur when species with staggered blooming periods support pollinator populations by reducing spatial and temporal variation in floral resource availability [50]. In this case, the consecutive bloom of plant species increases the duration of floral resource availability within years or the reliability of floral resources across years [51]. These same dynamics may be particularly important for pollinator communities in agricultural systems where crop rotation or extreme weather events can lead to high variability in floral resource abundance among seasons and years. If the greater abundance, diversity and duration of floral resources can be achieved through complementarity of flowering crops, later blooming crops such as strawberry may even support the pollination services of earlier mass flowering crops in the following year[52].

In agricultural systems, our findings reveal that crop habitats can act as a source of ecosystem services to other crops and represent an area of underexploited potential for ecological intensification practices. Studies of spillover of pollinators between mass flowering crops have also reported that prevalence of early mass flowering crops in the landscape can mitigate pollinator dilution in another mass flowering crops blooming in a later season [21]. Our results advance these findings by demonstrating that changes in the abundance of pollinators mediated by the bloom of mass flowering crops has consequences for the yield of nearby pollinator dependent crops. Importantly, our results highlight the importance of timing in determining the outcome of interactions among pollinator dependent crops and suggest ecological intensification strategies that may be employed to reduce competition and enhance facilitation among crops that have a significant number of shared pollinators. By selecting crops and varieties that bloom sequentially with shared pollinator communities, growers can minimize competition while maximizing facilitative effects, thereby improving the sustainability of crop pollination. However, when agronomic or other factors constrain variety selection, management strategies may focus on locating co-blooming crops at distances greater than the average foraging range of their shared pollinator community.

Conclusion

Our results clearly indicate that the timing of flowering in co-occurring crops can have consequences for the yield of pollinator dependent plants. When one crop co-blooms with another, mass flowering crop, competition for pollinators is likely to reduce yield, while flowering after the flowering event facilitates pollination leading to higher

yields. We show that the temporal resource pulses associated with mass flowering crops are an important driver of pollinator community dynamics and pollination services at local and landscape scales. Greater understanding of these effects will allow for improvements in designing agroecosystems in order to maximize the provisioning of ecosystem services and promote crop yields through ecological intensification.

Author Contributions

EB, KP and HG conceived of, designed and conducted the experiments. BD collected field data. HG and KP carried out the data analysis and drafted the manuscript. All authors approved the final manuscript.

Additional Information

Competing financial Interests

The authors declare no competing financial interests.

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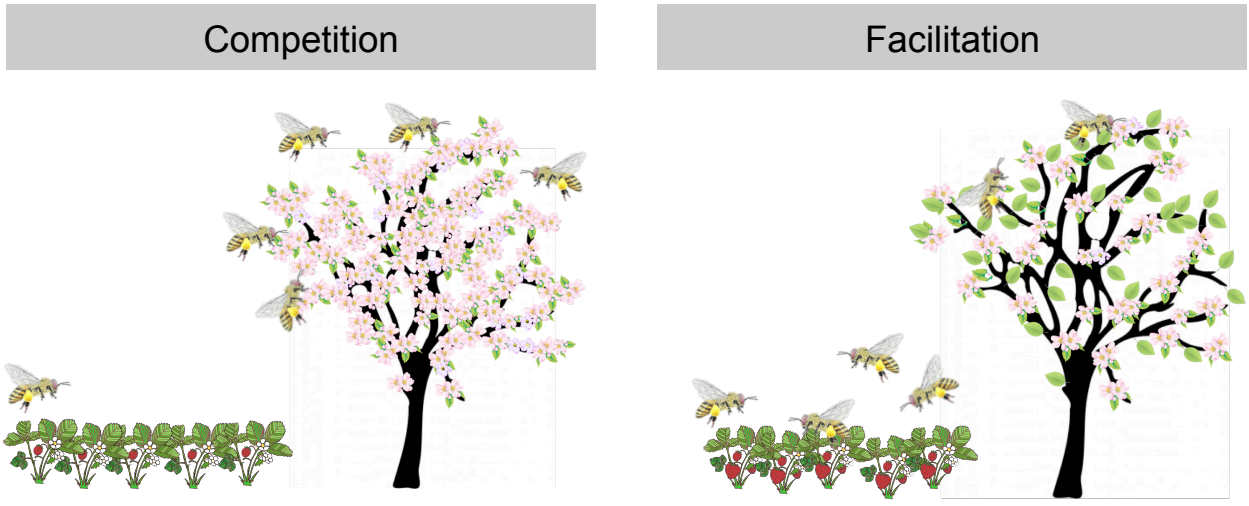

Figure 4.1. A simple conceptual model for the consequences of pollinator sharing between a mass flowering and co-blooming crop. A) Pollinator spillover from co-blooming crops to mass flowering crops during mass flowering results in competition for pollinators and a decrease in co-blooming crop yields. B) Pollinator spillover into co-blooming crops following bloom of mass flowering crops results in facilitation of pollinator visitation to co-blooming crops and an increase in co-blooming crop yields.

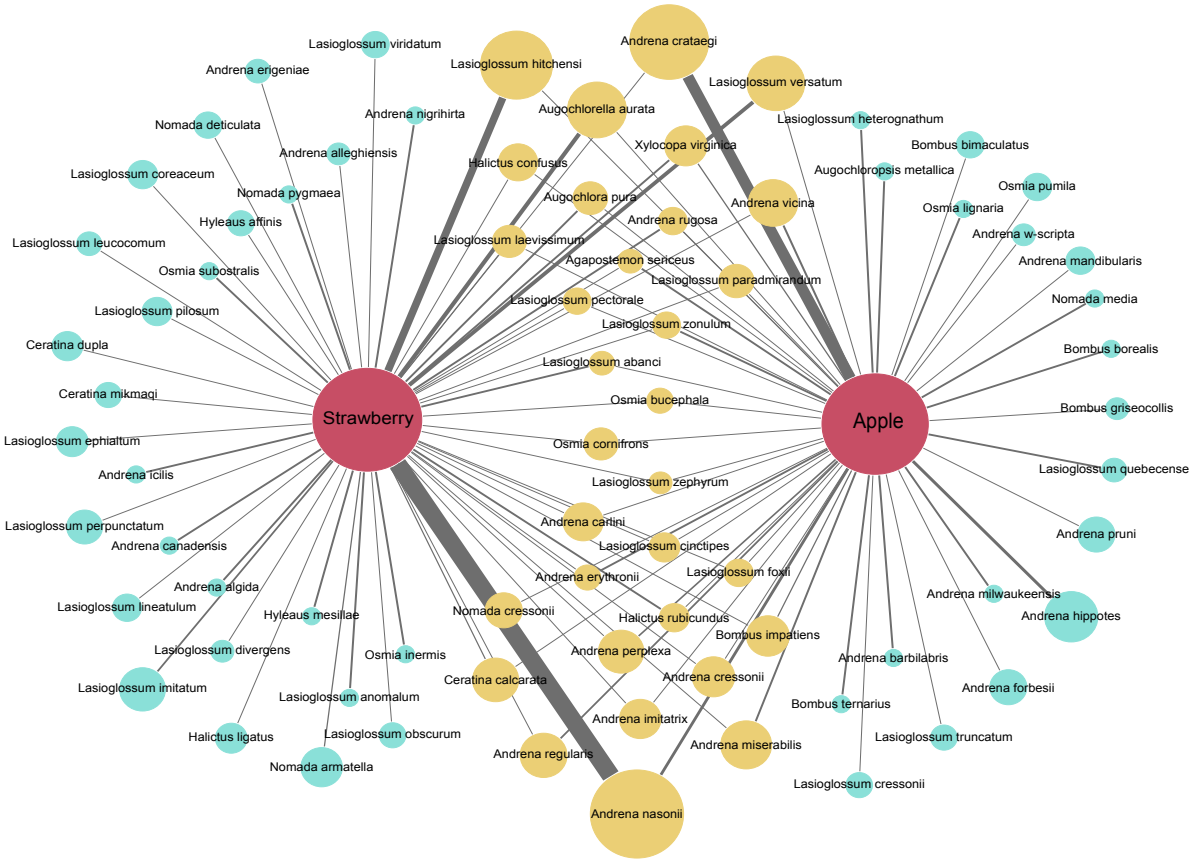

Figure 4.2. Pollinator communities of apple and strawberry in the Finger Lakes region of New York State. Node size indicates total abundance and edge widths represent relative abundance in each crop. Yellow=shared, Blue = unshared

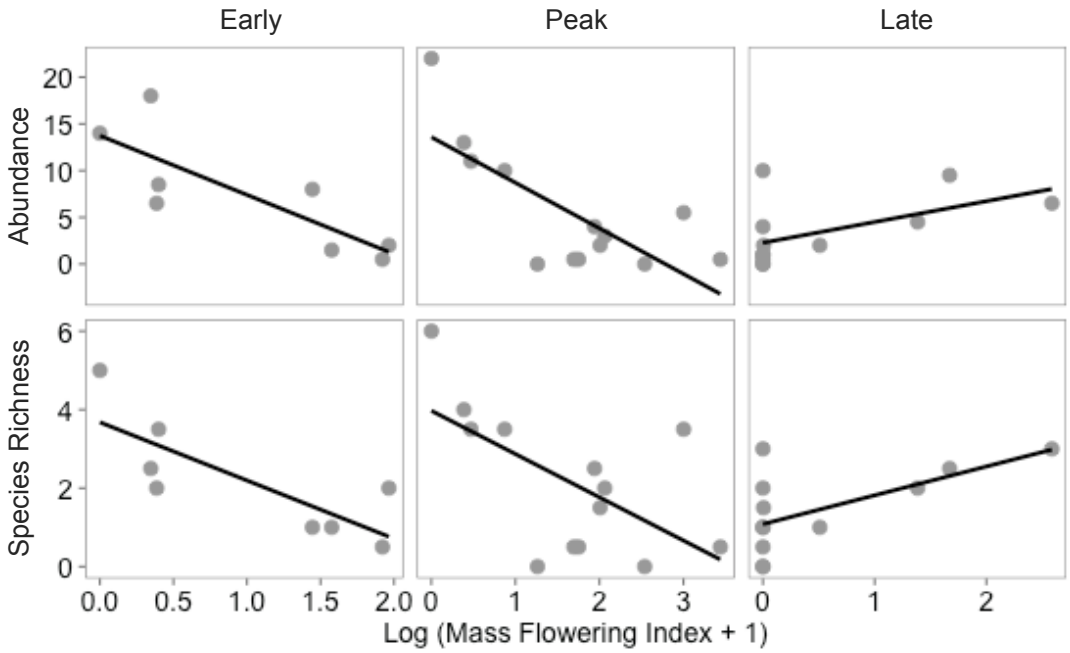

Figure 4.3. Average bee abundance and species richness during early, peak and late apple bloom in relation to the mass flowering index which describes the total amount of apple flowering within the surrounding landscape.

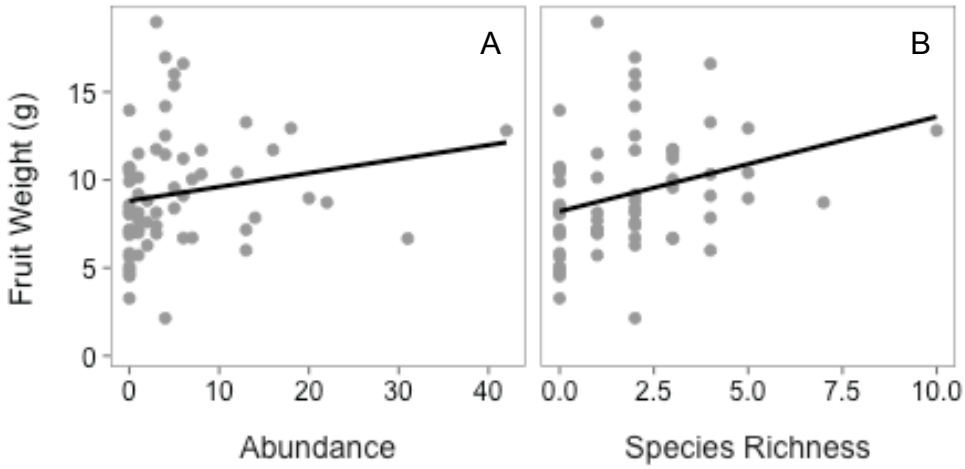

Figure 4.4. Averaged weight of strawberry fruits per farm relative to a) bee abundance b) bee species richness. Regression lines indicate significant relationships ($p<0.05$).

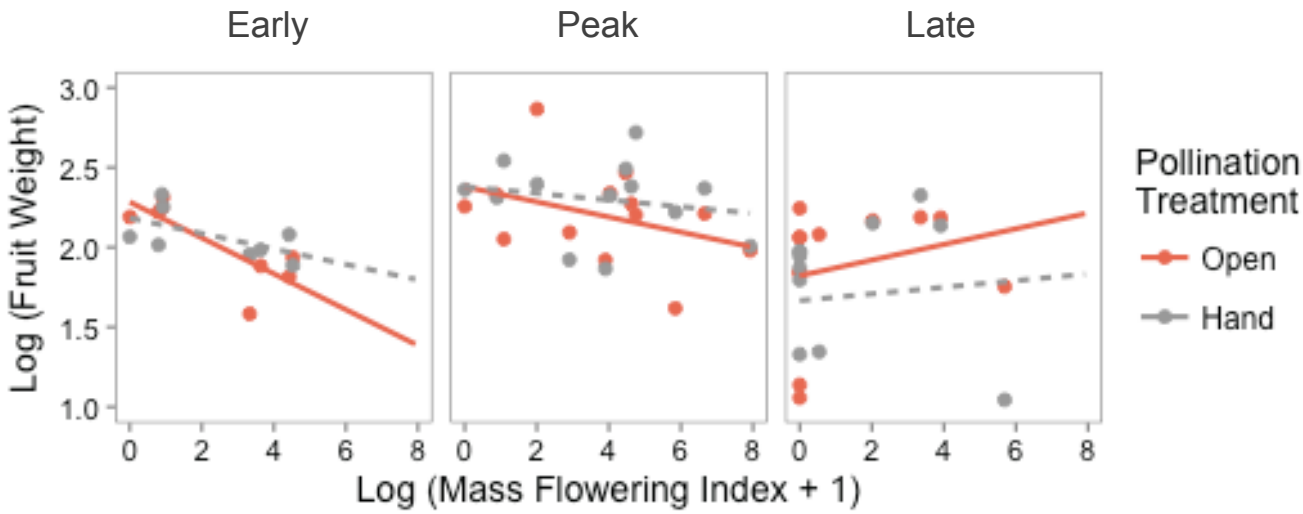

Figure 4.5. Average weight of hand-pollinated and open-pollinated strawberry fruits during early, peak and late apple bloom relative to the mass flowering index (calculated as the percentage of apple in the landscape multiplied by the intensity of apple bloom for each sampling period). Regression lines indicate significant relationships ($p<0.05$).

Table 4.1. Minimum adequate models describing local and landscape scale effects on abundance and species richness of bees in strawberry fields sampled during early, peak and late apple bloom from sites located adjacent or distant from natural areas.

Variable	df	*F*	*P*
Bee Abundance			
Stage	2,48	2.10	0.133
Natural Habitat Proximity	1,48	3.86	0.055
Mass Flowering Index	1,48	9.15	0.004
Stage X Index	2,48	8.41	0.001
Bee Species Richness			
Stage	2,49	0.94	0.394
Mass Flowering Index	1,49	2.14	0.149
Stage X Index	2,49	6.80	0.003

Table 4.2. ANOVA table output of minimum adequate models describing landscape scale effects of apple mass flowering on the weights of hand-pollinated and open-pollinated of sentinel strawberry fruits sampled during early, peak and late apple bloom.

Variable	df	*F*	*P*
Pollination Treatment	1,876	0.728	0.393
Flowering Stage	2,876	7.757	0.001
Mass Flowering Index	1,876	0.004	0.946
Poll. Trt. X Index	1,876	7.224	0.007
Stage X Index	2,876	8.322	0.001

CHAPTER 5

Landscape context mediates effectiveness of wildflowers for enhancing ecosystem services including crop yield

Abstract

Transition of natural habitat to agricultural use is one of the primary drivers of biodiversity loss worldwide. Landscape simplification associated with agricultural intensification threatens the delivery of ecosystem services that are supported by diverse communities of beneficial insects. The creation of flower rich habitats on agricultural lands has been promoted as a practice to support farmland biodiversity and enhance the delivery of ecosystem services. Few studies have evaluated their ability to support multiple ecosystem services simultaneously. Furthermore, theory suggests that the landscape context in which they are implemented will influence their success; yet, these predictions have not been empirically evaluated for ecosystem services, obscuring our ability to efficiently implement these practices in working landscapes. Here, we evaluate the impact of wildflower strip management on pollination, pest control, and crop yield across a landscape gradient. We find that wildflower plantings can increase pollinator visitation to adjacent crops but can also increase pest pressure. These effects depended on landscape context with the greatest benefits such as increased pollination, reduced pest pressure and increased production occurring in landscapes with intermediate cover of natural habitats. Thus, knowledge of landscape context can be used to target the implementation of wildflower plantings to areas where they will have the greatest likelihood for success with the least potential for increasing pest populations or decreasing yield in nearby crops.

Introduction

Diverse communities support many ecosystem services to agriculture, provide resilience to disturbances and maintain the capacity to adapt to future changing environments (1, 2). However, agricultural intensification undermines the very biodiversity and ecosystem services that would otherwise benefit crop production (3–5). Agricultural lands cover 40% of the earth's surface (6) and the continued transition of natural habitat to agricultural use is one of the primary drivers of biodiversity loss worldwide (7). Agricultural intensification at both local and landscape scales reduces the spatial and temporal availability of resources required by beneficial organisms, such as pollinators and natural enemies, while crop pests often benefit from a concentration of host plants (8).

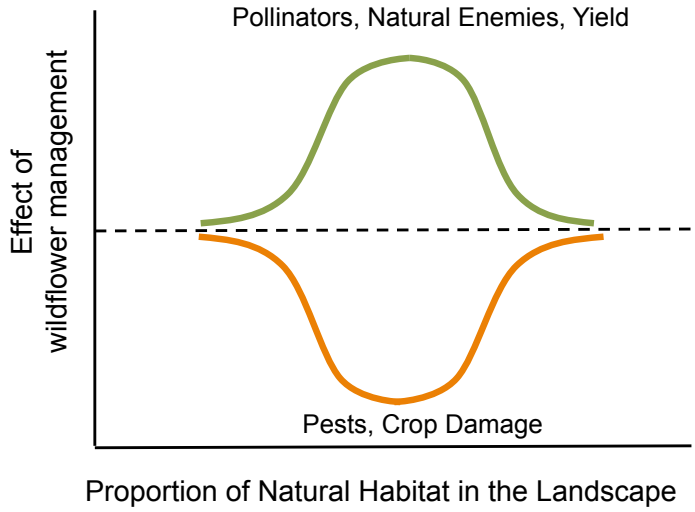

Figure 5.1. Conceptual diagram of nonlinear relationship between landscape composition and effectiveness of wildflower strip management in increasing pollinator visitation, natural enemy abundances and crop yield (green line) or reducing pest pressure and crop damage (orange line).

To increase agricultural sustainability, strategies are needed that reduce conflicts between biodiversity conservation and crop production. Ecological intensification capitalizes on the biodiversity within agroecosystems to achieve sustainable increases in crop yields by actively managing communities of ecosystem service providers (9, 10). Yet, a major hurdle to the widespread adoption of ecological intensification strategies is a framework for predicting the contexts in which they will be successful. Some inconsistencies in the performance of these practices may be due to the landscape context in which they are implemented. The intermediate landscape complexity hypothesis predicts that local management strategies will be most effective at improving biodiversity and ecosystem services when established in landscapes that are dominated by agriculture but with at least some natural habitat remaining (8, 11; Figure 1). In landscapes with high natural habitat cover, beneficial organisms continuously colonize agricultural habitats. Alternatively, source populations of beneficials are too depauperate to recruit from landscapes with very little natural habitat remaining, However, in landscapes with intermediate amounts of natural habitat, regional source populations are present, but agricultural habitats are not continuously colonized. Therefore, ecological intensification in landscapes of intermediate complexity is expected to produce the greatest effects and early findings from Europe support these patterns with respect to enhancing biodiversity (12–16). However, whether these finding are also reflected in the delivery of ecosystem services remains unresolved.

Multiple ecosystem services are expected to benefit from increases in local habitat diversity. For example, local management with flowering strips has been shown to

increase the abundance of pollinators and natural enemies of pests in adjacent cropland (16, 17). However, few studies have evaluated the effect of local habitat management on multiple services simultaneously (18), and even fewer have evaluated their combined impacts on crop yield (but see (19)). Pests can also benefit from natural habitats at the local and landscape scale (20, 21) thus management strategies aimed at increasing ecosystem services may fail to improve pest control or crop yield (22). Many governments and intergovernmental agencies have recently called for agricultural management practices that support biodiversity and ecosystem services on farms (White House Pollinator Protection Task Force 2016, IPBES 2017) making the need to ensure efficient placement and effectiveness more critical than ever.

In this study, we evaluate the benefits and potential costs of a commonly implemented ecological intensification strategy, the planting of native perennial wildflowers in field margins. We explore the effect of wildflower management on crop visitation by bees, biological control, pest abundance, crop damage and crop yield. Based on the intermediate landscape hypothesis, we predict that management with wildflower margins will improve ecosystem services and crop production to a greater extent when implemented in landscapes with intermediate amounts of natural habitat cover.

Methods

Study system

The experiment was conducted in the Finger Lakes Region of New York USA. The region is characterized by a diversity of agricultural uses, including dairy, row crop, tree

fruits and vegetables with natural and semi-natural areas of deciduous forest, small woodlots and old field dispersed throughout. In the region, strawberry (*Fragaria x ananassa*), is typically grown as a perennial with fields remaining in production between 4 and 6 years. Although strawberries are self-compatible and can be pollinated by wind, prior work in the same system has shown that pollination by insects, primarily bees, can increase yield by as much as 30% (23). Furthermore, the community of pollinators visiting strawberry is dominated by wild bees with more than 65 species regularly observed in a given year (23).

The primary insect pest of strawberry in the region is *Lygus lineolaris* (Hemiptera: Miridae). *L. lineolaris* are generalist herbivores that feed on the seeds of developing strawberry fruits. Feeding by *L. lineolaris* leads to developmental failure of the tissues around the seeds resulting in a characteristic “catfacing” type damage (24). Although overwintered adult females are present, they occur at low densities and the majority of feeding damage is done by the nymphs, which are the predominant life stage during the strawberry fruiting season (25). In the study region, the primary natural enemies of *L. lineolaris* include a complex of native and introduced parasitoid wasps in the genus *Peristenus* (26). Three species, *P. digoneutis* (introduced), *P. pallipes* (native) and rarely *P. relictus* (introduced), are known to attack *L. lineolaris*; however, parasitism rates are reduced in landscapes with a high proportion of agricultural land cover (Grab et al *in press).*

Experimental design

Within the region, we identified 12 farms within which we established two 10x15m plots consisting of 5 rows of strawberry (var. 'Jewel') in the spring of 2012. Plots were separated by a minimum of 200m and were randomly assigned to either a control border or a native perennial wildflower planting. Composition and management of control borders were representative of field edge management practices in the region. Control borders consisted primarily of orchard grass and were regularly mown over the growing season. Wildflower borders were 4m wide by 10m long and consisted of the following 11 US native perennial species *Zizia aurea*, *Penstemon digitalis*, *Coreopsis lanceolata*, *Potentilla fructosa*, *Vironicastrum virginicum*, *Agastache neptoides*, *Silphium perfoliatum*, *Lobelia siphilitica Solidago altissima*. These species were selected based on their attractiveness to bees and natural enemies (17, 27–30) and provide overlapping bloom periods so that flowers are present throughout the growing season. When possible every effort was made to grow plants from local ecotypes. Both border types were established in the fall of 2012. Plots were managed organically or involved limited use of pesticides for weed or fungal pathogen management. Each year, straw mulch was applied to all plots in the fall and raked into the row middles in the spring consistent with standard horticultural practices for strawberry in the Northeast.

At four plots it was necessary to prevent damage from large mammalian herbivores by erecting temporary plastic fencing. Fence gaps were wide enough (3 x 3 cm) to allow access to even the largest pollinators (HG pers. observation). In each case, both the control and wildflower treatment plots on the same farm were fenced.

Landscape

Landscape complexity was characterized using the National Agricultural Statistics Service Cropland Data Layer for New York (31) for each year of the study in ArcGIS 10.1. We quantified the cover of natural and semi-natural habitats at the 750m scale as previous studies of the pollinators, parasitoids, and pests in this system have found strong responses to this landscape metric at similar scales (23, 32); Grab et al *in press*).

Pollinator Surveys

In the three years following plot establishment (2013-2015), the abundance and diversity of the bee community was estimated by conducting visual surveys and sweep net sampling on four dates per plot spanning the duration of crop bloom. Surveys were carried out between 10:00 and 15:30 on sunny days with temperature above 16°C. Visitation rate was assessed using standardized 10min transects through each plot. Each visit was recorded, and each visitor was identified to the lowest taxonomic resolution possible on the wing (species or genus). The number of open strawberry flowers per sq ft was estimated for each plot by averaging counts of flowers in $1ft^2$ quadrats in each of the five rows. Visitation rates per plot were calculated by dividing the total number of visits recorded during the 10min transects by the average number of open flowers per sq ft.

To better understand the relative importance of the planted wildflower species, we monitored pollinator visitation rates to each species as well as visits to flowering weeds

within the border strips. All flowering plants within the border were observed for 10 min and total number of visits per species was recorded.

Pest Surveys

From 2013 to 2015 the abundance of *L. lineolaris* was estimated in each plot immediately following strawberry flowering by tapping individual strawberry inflorescences until a total of 24 nymphs were collected or all inflorescences in the plot were sampled. We choose a target of 24 nymphs per sample because this number allowed us to accurately estimate parasitism rates using the protocols described below. Nymph densities were calculated by dividing the number of nymphs collected by the total number of inflorescences tapped.

Because wildflower plantings may harbor pest populations that can spill over into the crop, we estimated the abundance of *L. lineolaris* in the wildflower plantings compared to the control borders for an entire growing season in 2015. The abundance of *L. lineolaris* adults and nymphs was estimated for each flowering species present in the wildflower plantings by vacuuming (Echo ES 230 Shred 'n Vac, Lake Zurich, IL) 25 inflorescences of each plant species once a week from May to October. Plants were sampled at the bud, flowering and seed head phases so that our estimate for each species accurately reflect the broad feeding preferences of *L. lineolaris*. After sampling a particular species, all *L. lineolaris* were returned to host plant they were collected from to ensure that the effects of sampling in one week had little impact on samples in the subsequent weeks. Sampling also included any weedy flowering species that had

invaded the perennial plantings. As some plant species had fewer than 25 inflorescences on any particular sampling date, the total number of *L. lineolaris* collected was divided by the number of inflorescences vacuumed for each sample. The order of sampling was randomized for species blooming on a given date. An equivalent number of vacuum samples were obtained from the grassy margins of control plantings for each wildflower species sampled from it's paired wildflower treatment plot.

Parasitism Rates

Diagnostic PCR assays were used to simultaneously estimate parasitism rates and parasitoid species identity, as they are faster and more accurate than rearing or dissection (33, 34). Random samples of 24 nymphs from each sampling period at each site were selected for parasitism assays. In some cases, fewer than 24 nymphs were collected in a sampling period. In these cases, all collected nymphs for the period were processed. DNA from nymphs was extracted using an abbreviated chloroform: isoamyl alcohol protocol developed by (35). DNA extractions along with negative controls were amplified using *Peristenus* species-specific primers as in (36). Using this method, species-specific forward primers are combined with a genus-specific reverse primer to amplify a region including ITS1 and ITS2. The presence of an amplicon indicates parasitism and the length of the fragment indicates parasitoid species identity.

Fruit Damage and Yield

Strawberries are an aggregate accessory fruit comprised of as many as 300 achenes on a primary fruit and 200 on a secondary fruit (37). Each achene must be fertilized for

the surrounding tissue to develop and an average of four visits per flower is required to achieve full pollination (38). *L. lineolaris* nymphs and adults feed on developing achenes leading to a failure in development of the surrounding tissues. The weight of a fruit is highly correlated with the number of developed undamaged achenes (37). Fruits with a high percentage of damaged achenes, either from poor pollination or *L. lineolaris* feeding, develop with major malformations that reduce overall yield and marketability (39).

To measure the impact of wildflower plantings on crop yield at each site, 30 secondary fruits from each plot were harvested when ripe and weighed. The percentage of poorly pollinated and damaged achenes was estimated for each fruit. A typical strawberry inflorescence is comprised of a single primary fruit (king berry), a pair of secondary fruit, and four tertiary fruit. Secondary fruits were used, as they are less prone to frost damage than primary fruit and due to their later development are more susceptible to damage from *L. lineolaris* nymph feeding.

Statistical Analysis

To evaluate whether wildflower strip management had differential effects across the landscape gradient, we calculated an index of wildflower management effectiveness for each variable by subtracting the average value observed on the plot with a wildflower border minus the control divided by the control ((WF-C)/C) of each site in each year. We then constructed linear and non-linear mixed effects models for each index (GLMER, R package lme4, 40) with Gaussian error structures. Fixed effects in each model included

year and proportion of combined natural and semi-natural habitat cover as well their interaction. We constructed linear, logistic, and polynomial models for each variable and selected the best fit model based on AICc values. Farm was included as a random effect in each model to account for repeated measures across years.

Differences in *L. lineolaris* numbers and bee visits to flowering species within the plot margins and between wildflower and control margins were assessed in generalized linear mixed effect models. For both variables, plant species was included as a fixed effect and farm was included as a random effect. For overall *L. lineolaris* abundance in wildflower compared to control borders, an index was computed similar to those described above. Fixed effects included year and proportion of combined natural and semi-natural habitat cover as well their interaction.

Results

In total, 5,684 bee visits were recorded over the three years of surveys and 1,307 bee specimens were collected. Wild bees were the dominant visitors, representing 95.8% of the community while managed bees (honey bees) made up only 4.2% of recorded visits. In total, 99 species were recorded based on net collected specimens. A total of 3,197 *L. lineolaris* nymphs were collected from the strawberry plantings over the three years of the study. Parasitism assay revealed an 18% overall parasitism rate. Three parasitoid species were detected with the introduced *P. digoneutis* being the dominant natural enemy (96.7% of parasitism events) and the other two species, *P. pallipes* (native, 2.8%) and *P. relictus* (introduced, 0.05%), represented at low levels.

Interestingly of the 12 occurrences of *P. pallipes* parasitism, the nymph was also identified as being positive for *P. digoneutis* parasitism in 8 of the cases. Wildflowers bloomed from April to November each year. On average 7 of the 9 wildflower species became established at each site but no site had fewer than 6 species.

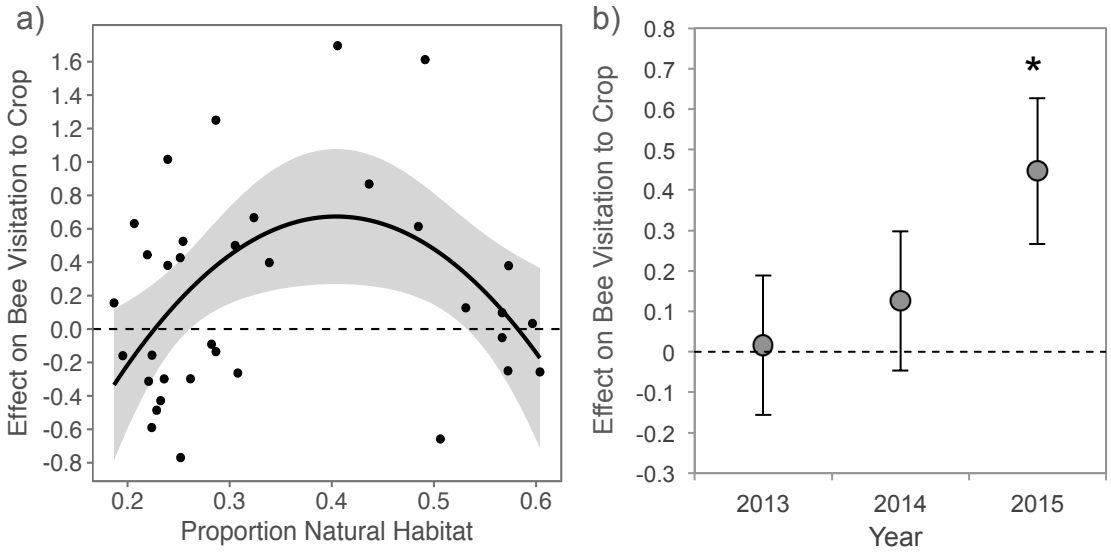

Figure 5.2. Effectiveness of wildflower strip management relative to control plots ((wildlfower-control)/control) for bee visitation to strawberry flowers a) in relation to the proportion of natural land cover in a 750 m radius around each site across all three years of the study and b) in each of the study years following wildflower establishment in 2012. Asterisk in b) indicates value different from 0 at $P < 0.05$ based on post-hoc contrast tests. Shaded areas in a) represent 95% confidence intervals.

The effectiveness of wildflower strips for increasing bee visitation across the landscape gradient was best described by a second order polynomial function ($AICc_{poly} = 63.86$, $AICc_{log} = 73.86$, $AICc_{linear} = 73.89$; *Poly*: $F_{(1,21)} = 7.33$, $P = 0.01$). Wildflower strip management increased bee visitation to strawberry relative to controls only in

landscapes with intermediate amounts of natural habitat (Fig. 2a). On average, wildflower borders had little effect on bee visitation in the first two years after establishment, but had positive effects in 2015 ($t_{(1,21)}$ = 2.48, P = 0.02; Fig. 2b).

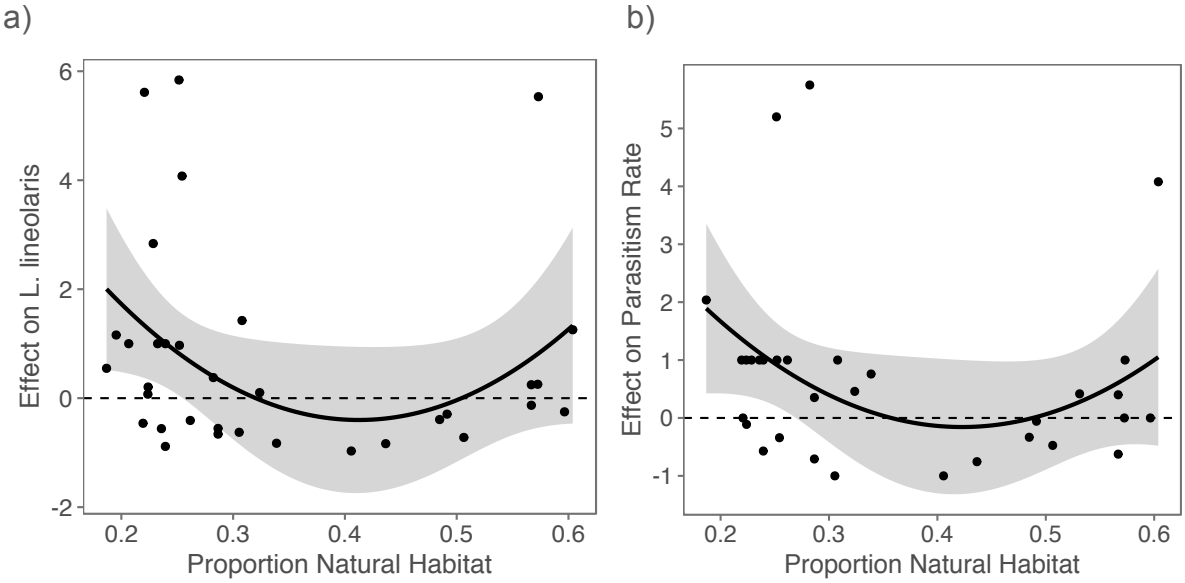

Figure 5.3. Effectiveness of wildflower strip management relative to control plots ((wildflower-control)/control) for a) the number of *L. lineolaris* nymphs and b) the parasitism rate of nymphs. Shaded areas represent 95% confidence intervals.

The effect of wildlfower strip management on *L. lineolaris* abundance was marginally influenced by the landscape according to a second order polynomial function ($AICc_{poly}$ = 127.7, $AICc_{log}$ = 136.3, $AICc_{linear}$ = 136.1; *Poly*: $F_{(1,18)}$ = 3.71, P = 0.06). Pest abundances on plots with a wildflower border were greater than controls in the landscapes with the least and most natural habitat cover (Fig. 3a). In intermediate landscapes, wildflowers borders decreased pest pressure below the levels of control plots. *L. lineolaris* abundance on plots with a wildlfower border differed across the years

($F_{(2,18)}$ = 7.88, P = 0.003; Figure S1) and was greatest in 2014 ($t_{(1,21)}$ = 4.67, P < 0.001).

The effectiveness of wildflower management across the landscape gradient on parasitism largely mirrored the pattern observed for pest abundances (Fig. 3b). Parasitism rates were strongly positively correlated with the abundance of L. lineolaris nymphs ($F_{(1,59)}$ = 8.17, P = 0.005). Again a polynomial function best fit the data ($AICc_{poly}$ = 117.7, $AICc_{log}$ = 126.5, $AICc_{linear}$ = 126.4; *Poly*: $F_{(1,16)}$ = 4.06, P = 0.06). However, parasitism rates followed a pattern across years similar to bee visitation; achieving the highest values on wildflower plots relative to controls in 2015 ($t_{(1,18)}$ = 2.48, P = 0.02; Figure S2).

Sampling *L. lineolaris* within the plot margins themselves revealed that densities of *L. lineolaris* were significantly higher in wildflower plantings compared to control borders throughout the season (WF: $F_{(1,10)}$ = 30.47, P = 0.0003). Although there were no significant differences in the number of *L. lineolaris* collected in control margins between the landscape types, wildflower margins in landscapes with intermediate natural habitat cover supported significantly greater numbers of *L. lineolaris* in comparison to control borders (Figure 4a, $F_{(1,10)}$ = 5.42, P = 0.052). The number of *L. lineolaris* supported by different species of wildflowers varied ($F_{(12,238)}$ = 1.94, P = 0.03; Figure 4b) as did the number of bee visitors to each species ($F_{(14,86)}$ = 8.12, P , 0.0001; Figure 4b).

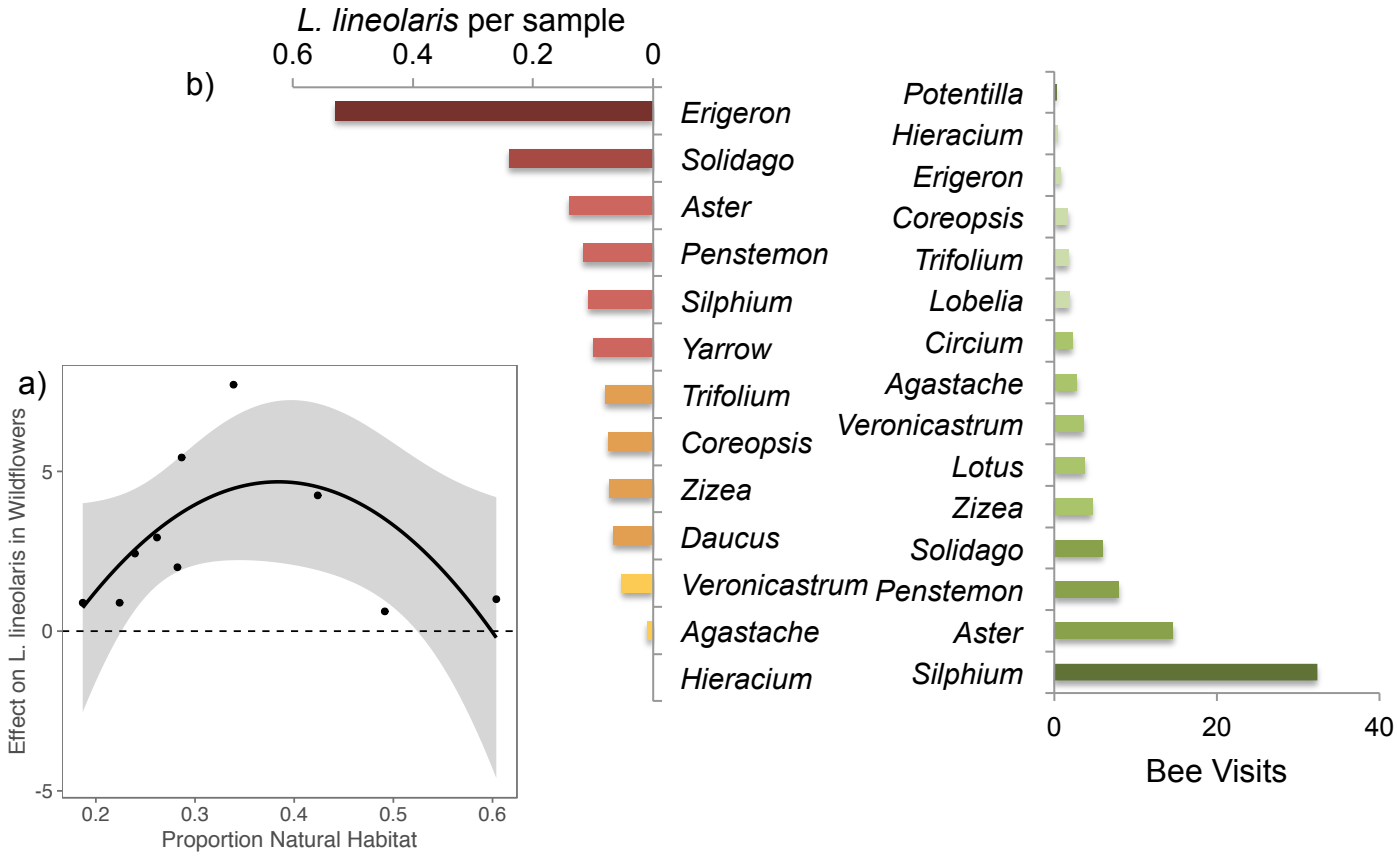

Figure 5.4. The number of *L. lineolaris* nymphs collected within the wildflower plantings a) relative to control plantings and b) on various wildflower species relative to the number of bees visiting each wildflower species.

Both lack of pollination by bees and feeding damage by *L. lineolaris* cause damage to developing strawberry fruit resulting in yield loss. The relative importance of *L. lineolaris* abundance vs. bee visitation in predicting damage varied across study years (TPB x Year: $F_{(1,11)}$ = 36.03, P < 0.001; Bee x Year: $F_{(1,11)}$ = 33.26, P < 0.001). In 2013 and 2014, *L. lineolaris* abundance was the only significant predictor of fruit damage and increasing nymph abundance was associated with greater damage (2013 TPB: z = 2.98, P = 0.002, Bee: z = 0.22, P = 0.823; 2014 TPB: z = 2.17, P = 0.029, Bee: z = -0.21, P = 0.829). In 2015, both groups predicted damage; although, bee visitation had a stronger effect (Bee: z = -2.74, P = 0.006; TPB: z = 2.24, P = 0.025; Figure S3) consistent with increasing positive effect of wildflowers on bees over the three-year study.

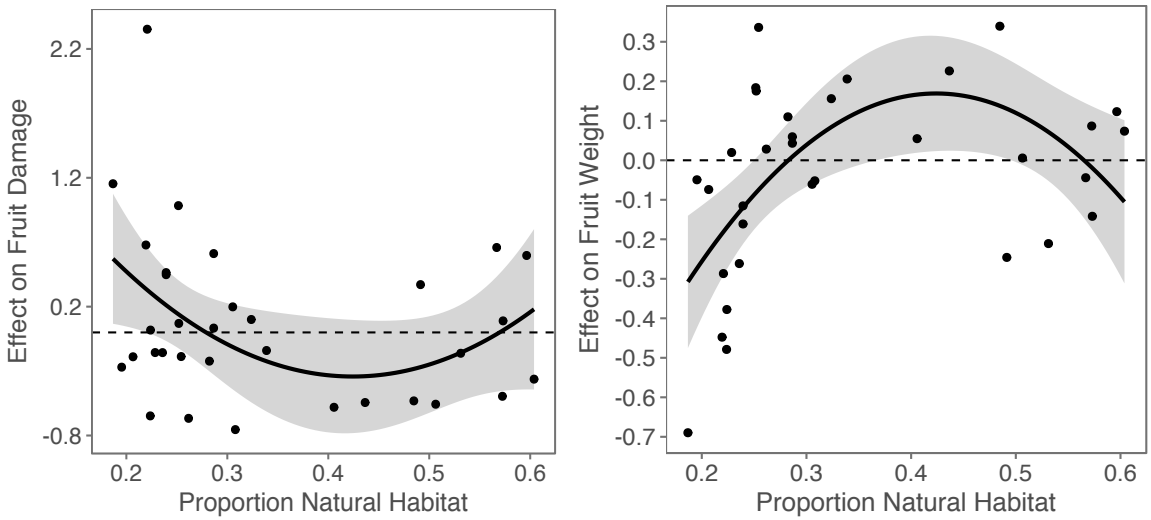

Figure 5.5. Effectiveness of wildflower strip management relative to control plots ((wildflower-control)/control) for a) the damage to and b) the weight of strawberry fruits. Shaded areas represent 95% confidence intervals.

The difference in fruit damage on plots with a wildflower border compared to controls was best explained by a ploynomial response to landscape ($AICc_{poly}$ = 65.83, $AICc_{log}$ = 72.35, $AICc_{linear}$ = 72.58; *Poly*: $F_{(1,19)}$ = 3.48, P = 0.07). Damage caused by both poor pollination and *L. lineolaris* feeding was greatest on plots with a wildflower border in landscapes with the least natural land cover (Fig 5a). Landscapes with intermediate cover of natural habitat had the greatest reduction in damage, however wildflower plots had increasingly more damage relative to controls as the proportion of natural land cover increased from intermediate levels.

For fruit weight, a polynomial function also the best fit for the relationship between wildflower strip effectiveness and landscape ($AICc_{poly}$ = -0.33, $AICc_{log}$ = 7.42, $AICc_{linear}$ = 8.52; *Poly*: $F_{(1,19)}$ = 8.68, P = 0.008). Plots with a wildflower border had higher yields than controls in landscapes with intermediate amounts of natural land cover (Fig 5b). The difference between wildflower and control plantings decreased in landscapes with the least and the most natural cover.

Discussion

The creation of flower rich habitats on agricultural lands has been promoted as a practice to support farmland biodiversity and promote the delivery of ecosystem services (White House Initiative, EU Initiative, IPBES report 2016, 11, 29, 41–43). Yet, few studies have evaluated the effectiveness of these plantings across a gradient of landscape contexts or on multiple ecosystem services simultaneously, impeding our

ability to determine if practices targeted for the conservation of one ecosystem service will impact the delivery of other services. Here, we evaluate the impact of wildflower strip management on pollination, pest control and crop yield across a landscape gradient.

Bee visitation to crop flowers increased with the addition of local wildflower plantings in landscapes with intermediate cover of natural habitats. This pattern was best described by a polynomial function as predicted by the intermediate landscape hypothesis (8, 11). Interestingly, the intermediate values of land cover that correspond with success of the wildflower plantings are shifted strongly towards higher values compared to those originally proposed by Tscharntke et al in European landscapes. Tscharntke et al proposed that wildflower plantings would have the strongest effects in landscapes with 1-20% non-crop habitat. In our study, wildflower habitats were the most successful in landscapes with 25-55% natural habitat cover. These differences in threshold values likely reflect the differences in the composition of the current dominant natural habitat covers (grasslands in Europe, forest in the northeastern US) and the differences in the history of large-scale agricultural land use between the regions (thousands of years in Europe, hundreds in the northeastern US). Indeed, the effectiveness of supplementing floral resources for enhancing parasitism rates in California vineyards was greatest when landscapes contained 20-60% natural habitat (44). These results imply that polices attempting to prioritize areas for conservation and ecosystem services management need to be tailored as the response curves for other areas of the globe will likely differ from those observed in this study.

For pest pressure, the shape of the relationship between landscape and effectiveness is also predicted by the intermediate landscape hypothesis, yet the curve is shifted strongly above the mean. This shift represents a cost of wildflower management not predicted by the intermediate landscape hypothesis. In landscapes with the least and greatest natural habitat cover, plots with a wildflower border had greater pest abundances than those with a control border. Although flowering border plantings are intended to target beneficial insects, generalist pests like *L. lineolaris* are also able to take advantage of these additional resources (17, 20). In highly agricultural landscapes, *L. lineolaris* accumulate at higher numbers in wildflower borders compared with landscapes with more natural habitats. This result likely reflect the lower propensity for *L. lineolaris* to disperse in agriculturally dominated landscapes (45) and may lead to increased spillover of pests from the wildflowers to the crop in the following spring.

The relationship between landscape and effectiveness for parasitism was opposite of our predictions based on the intermediate landscape hypotheses. Rather, wildflower plots with the greatest increases in parasitism relative to controls were in the same landscape contexts that also had the greatest relative increases in pest abundances. Therefore, parasitoid responses to wildflower management may be obscured by density dependent responses to host abundance (46). However, other studies have found positive effects of wildflower management on biological control of pests (17, 47), particularly when the pest was not able to use the flowering strip for alternative hosts.

The lag in time between the establishment of wildflower plantings and the response of the beneficial insect community can influence the cost-benefit ratio for farmers implementing these plantings with the goal of enhancing ecosystem services (28). In our study, increases in bee visitation and parasitism rates occurred in the third year following establishment. Although a number of studies report responses within the first year following establishment (16, 17, 30, 48) the majority of these studies report on communities within wildflower plantings rather than in adjacent crop habitats (16, 30) while others use annual plants in their borders (48). Many studies evaluate the effects of wildflower plantings on bee visitation or natural enemy communities but few assess the impact on crop damage and the final effect on yield. In our study, wildflower management tended to increase fruit damage and reduce yield in landscapes with low natural habitat cover. In these same landscapes, wildflower management had little effect on bee visitation and increased pest abundances. In landscapes with intermediate natural habitat cover, plots with a wildflower border had less damage than those in simple landscapes again corresponding with patterns in pollinator and pest responses. These results highlight that wildflower management can lead to greater delivery of ecosystem services and enhanced crop yield in some landscape contexts. These effects are primarily driven through enhancement of pollination rather than biological control services.

Our study highlights that wildflower strip management is not without costs imposed by increased herbivore pressure. Yet increases in herbivore pressure were only observed in landscapes where wildflower strips had the least success in improving bee visitation.

In all landscape contexts, efforts should focus on selecting wildflower species that are not preferred by crop pests and on managing weedy hosts. Management for these weedy species can also increase the establishment rates of planted species (49). In very simple landscapes where wildflower management has few benefits, efforts should focus on the conservation of the remaining natural habitat and restoration of larger natural areas.

Because of the importance of landscape in mediating the success of wildflower plantings, we propose that landscape context should be explicitly considered in large policy initiatives that subsidize the creation of flowering habitats on farmlands. By implementing these metrics, limited resources for establishing habitat for beneficial insect conservation can be targeted to areas where they will have the greatest likelihood for success with the least potential for increasing pest populations or yield loss in nearby crops.

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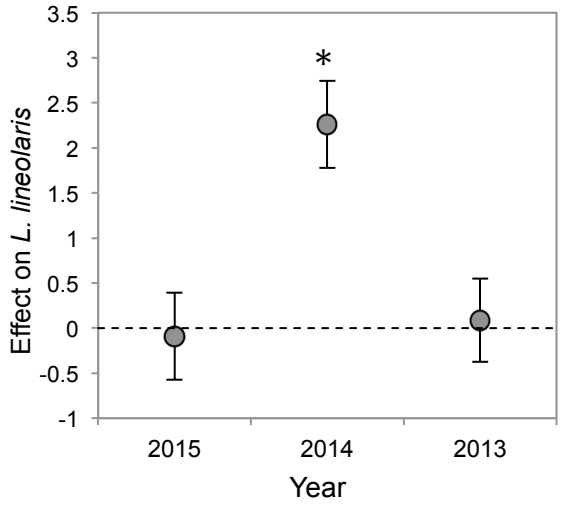

Figure 5.S1. Average effect of wildflower management on *L. lineolaris* in each of the study years following wildflower establishment in 2012. Asterisk indicates value different from 0 at $P < 0.05$ based on post-hoc contrast tests.

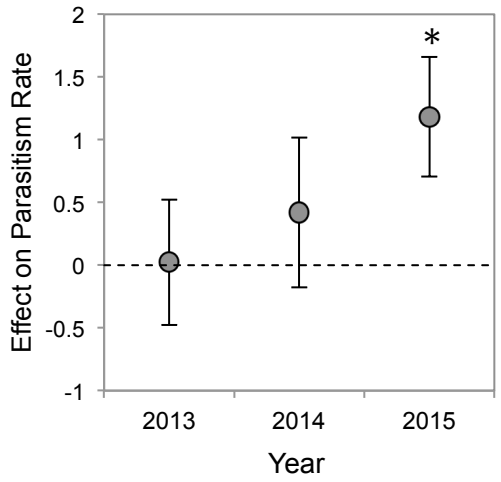

Figure 5.S2. Average effect of wildflower management on parasitism rates of *L. lineolaris* nymphs in each of the study years following wildflower establishment in 2012. Asterisk indicates value different from 0 at $P < 0.05$ based on post-hoc contrast tests.

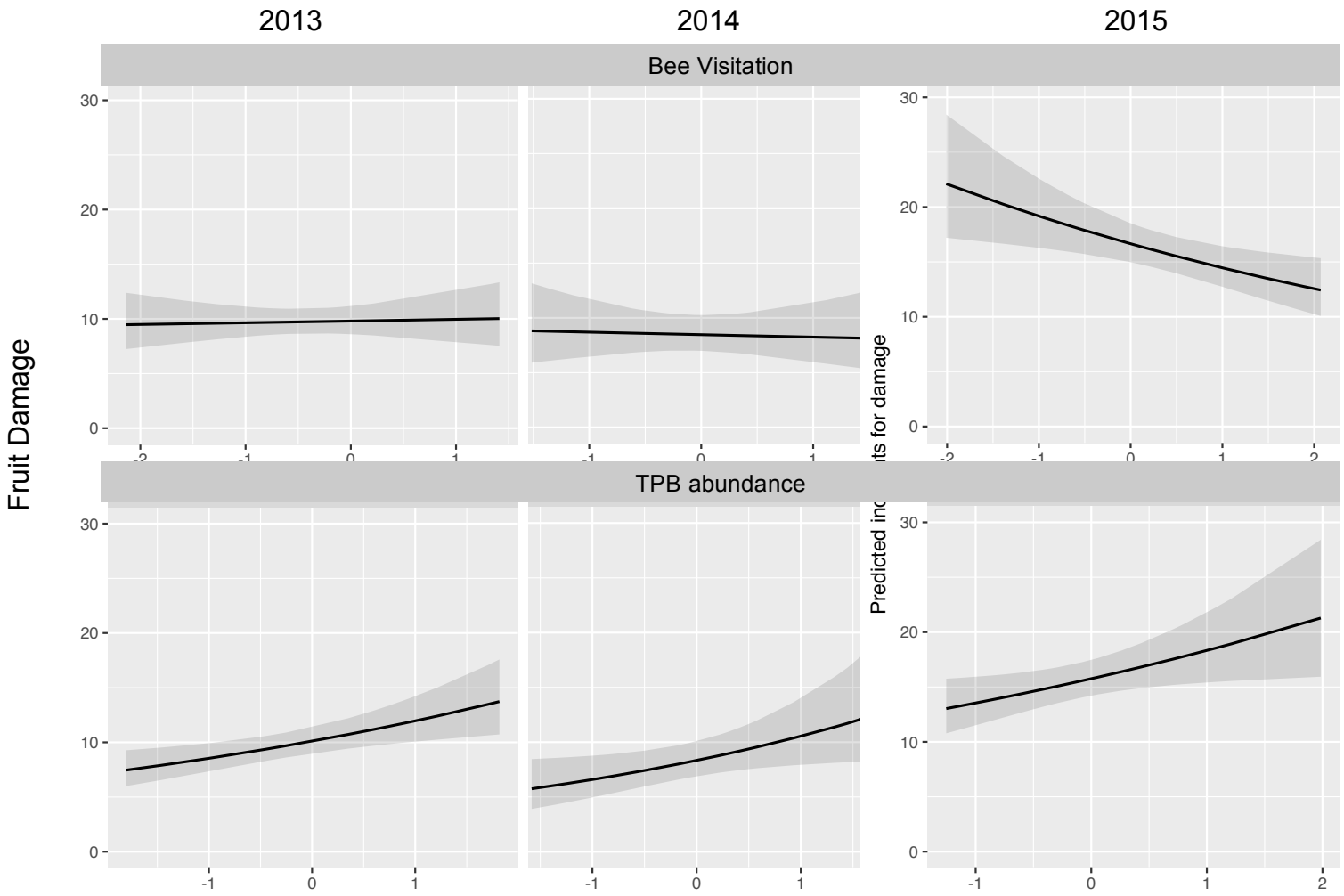

Figure S3. Effect of both bee visitation (top panels) and *L. lineolaris* abundance (bottom panels) on damage to strawberry fruit in each of the three years of the study.